# LINKING GERMINATION TRAITS OF OILSEED RAPE (*Brassica napus* L.) TO DNA MARKERS

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A thesis submitted in partial fulfilment of the

requirements of the University of Abertay Dundee

for the degree of Doctor of Philosophy

May 2000

I certify that this thesis is the true and accurate version of the thesis approved by the examiners.

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## ABSTRACT

Oilseed rape has become an important weed in arable rotations and a feral plant of field margins, soil dumps and roadsides. Seed persistence in the soil following induced secondary dormancy is thought to perpetuate these weed and feral populations. Potential variation between cultivars has been suggested in previous work in the extent of secondary dormancy and other germination traits, and in underlying genetic heterogeneity. The aim of this work was to quantify in more detail inter- and intra- cultivar variation in germination traits for six oilseed rape cultivars, to confirm that variation was consistent in laboratory and field, and to ascertain whether there is a genetic base to this variation. The cultivars are Askari, Bristol, Gazelle, Libravo, Martina and Rocket, selected on the basis of their suspected heterogeneity.

Laboratory germination tests were conducted at 4°C, 10°C and 19°C on a thermal plate and confirmed substantial inter-cultivar variation in germination rate, induction of secondary dormancy and the temperature stimuli required for dormancy break. The phenotypic traits were quantified by mathematical parameters and cultivars ranked in order of decreasing heterogeneity.

DNA analysis was made on leaf tissue of early, middle and late germinating phenotypes using two simple sequence repeat primers. There was heterogeneity and phenotypic variability generally, but a direct association between phenotype and genotype was found only in the cultivar Martina.

Field emergence trials revealed non-linearity in emergence and a strong similarity between laboratory germination and field emergence curves. Cultivar heterogeneity was found to be similar for emergence rate and post-winter emergence. Again there was evidence of an association between heterogeneity in emergence and genetic heterogeneity in the DNA markers.

The results suggest that standard seed testing should be carried out at low temperatures to detect any hidden variability in germination. Plant breeders should be cautious about introducing variability into new breeding lines as this might increase the potential persistence of feral populations and the risk of gene transfer to later

**TABLE OF CONTENTS** **Page**

## LIST OF TABLES

## LIST OF TABLES (Continued)

## LIST OF PLATES AND ILLUSTRATIONS

## LIST OF PLATES AND ILLUSTRATIONS (Continued)

## LIST OF ABBREVIATIONS

| | |
|---|---|
| A | Adenine |
| Abs. | Absolute |
| AFLP | Amplified fragment length polymorphism |
| Bp | Base pair |
| C | Cytosine |
| CAP | Common Agricultural Policy |
| cv. | cultivar |
| dATP | 2′-Deoxyadenosine 5′ triphosphate |
| dCTP | 2′-Deoxycytidine 5′ triphosphate |
| dGTP | 2′-Deoxyguanosine 5′ triphosphate |
| dTTP | 2′-Deoxythymidine 5′ triphosphate |
| DNA | Deoxyribonucleic acid |
| EDTA-$Na_2$ | Ethylenediaminetetra-acetic acid disodium salt |
| EEC | European Economic Community |
| Glogistic | Generalised logistic |
| GMO | Genetically modified organism |
| IAA | Isoamyl alcohol |
| M | Molar |
| mM | Millimolar |
| $MgCl_2$ | Magnesium chloride |
| NaCl | Sodium chloride |
| NIAB | National Institute of Agricultural Botany |
| OECD | Organisation for European Co-operation and Development |
| OSR | Oilseed rape |
| PCO | Principal Co-ordinates |
| PCR | Polymerase Chain Reaction |
| RAPD | Randomly amplified polymorphic DNA |
| Rep | Replicate |
| RFLP | Restriction fragment length polymorphism |
| Rnase A | Ribonuclease A |
| SCRI | Scottish Crop Research Institute |
| SDS | Sodium dodecyl sulphate |
| SSR | Simple sequence repeat |
| T | Thymine |
| TDR | Time domain reflectrometry |
| TE | Tris-EDTA |
| Tris | Trishydroxymethylaminomethane |
| Tris-HCl | Tris-Hydrochloride |
| µl | Microlitre |
| w/v | Weight/volume |

## ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. B. Marshall and Dr. G. Squire at the Scottish Crop Research Institute for their great support and professionalism. I would also like to thank my director of studies Prof. K. Gartland at the University of Abertay Dundee. A thank you to the Scottish Crop Research Institute for allowing this project to be carried out under work funded by the Scottish Executive Rural Affairs Department.

## DECLARATION

I hereby declare that the work contained in this thesis is the result of my own investigation, except where reference has been made to published literature. I also declare that this work has not already been accepted in substance for any degree.

Signed .....Gordon Dunlop.....

Date .16/3/2001.....

# Chapter 1

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 INTRODUCTION

In recent years, oilseed rape has become an important weed in arable rotations and a feral plant of field margins, soil dumps and roadsides. These feral colonies are established through seed spillage from mechanised agricultural transport or natural carriers such as birds and mammals. The progeny from the feral plants creates seedbanks that are annually regenerative enabling the survivorship of the colony for several years, recent evidence has shown that some seedbanks can persist for 5 years (Charters, 1996) and circumstantial evidence points to ten years. Weeds and feral populations are attracting interest because they are potential channels for gene exchange between transgenic and normal crops in agricultural use (Becker *et al.*, 1992; Jorgensen *et al.*, 1994).

Oilseed rape, being partially allogamous, has an average outcrossing rate of between 20% and 40% and as modern agricultural practices are demanding the use of genetically modified cultivars to solve their problems, there is an increasing risk of escaped transgenes spreading through pollination to other cultivars and wild species (Timmons *et al.*, 1995; Linder & Schmitt, 1995).

The market for vegetable oil requires cultivars to have a low erucic acid content and the animal feed industry requires a low glucosinolate content to ensure a high standard product. Contamination by gene transfer from an undesirable cultivar would reduce the quality of the crop.

There is little knowledge, however, of the dynamics of these populations (Pekrun *et al.*, 1996) and whether cultivars differ in their propensity to become weedy.

Seed dormancy, survivorship and germination cues determine the potential ecological invasiveness of a cultivar due to the ability of seed to exploit time-windows in the annual cycles of weather and cultivation (Adler *et al.*, 1993).

The commercial pressures driving the usage of genetically modified cultivars are also creating debate and diverse public opinion on ecological and ethical grounds. Many questions are being asked on the issues of risk assessment of transgene escape and its long-term implications for the environment, these answers must be met comprehensively in order to fully satisfy public confidence.

Cultivar differences by definition are genetically based giving rise to phenotypic variation. However, identification on a morphological basis alone is extremely difficult. Plant breeders are producing new cultivars with only minor morphological but major metabolic differences from their parents, eg lower erucic oil content, which cannot be identified by appearance alone. There are many technological methods for identifying differences in specific traits resulting in cultivar identification, but the success in some of the techniques depends on the degree of dissimilarity between the cultivars. Modern molecular biological techniques can now enable accurate inter- and intra-cultivar identification based on its genetic signature due to having a large number of polymorphic microsatellites in its genome.

### 1.1.1. Project aims

The overall aim of this research is to investigate the inter-and intra-cultivar variation in the germination characteristics of oilseed rape in response to temperature, determine the genetic banding profiles generated by SSR primers, link phenotype with genotype, and consider the potential implications for possibility of gene transfer and survivorship in a feral colony.

Specific attention will be given to:

- The non-germinating trait observed in some cultivars being genetically determined.
- The quantitative rate of germination, as a heritable characteristic.
- The non-germinating and the rate of germination traits are responsible for the variation in emergence over winter.
- The comparison of germination responses between controlled laboratory and natural environmental conditions.
- The comparison of the genetic variation within oilseed rape cultivars.

## 1.2 LITERATURE REVIEW

### 1.2.1 OSR Origins & Agricultural Production

Oilseed rape is an amphidiploid of the species *Brassica* and evolved through the hybridization of *B. oleracea* and *B. campestris* to form the sub-species *Brassica napus* (Song & Osborn, 1992) and is thought to have originated in the mediterranean region.

The cultivation of *Brassica napus* L. on a commercial scale in Europe probably occurred around the 13th century A.D. when its principal use then was as a lamp oil. In the U.K., the Commonwealth was the traditional supplier of raw seed for vegetable oils, but supplies became increasingly difficult to obtain as the supply countries developed their own refining and production facilities. The demand for self-sufficiency in vegetable oils during the mid-nineteen sixties, and the added benefit to farmers of having a good weed control when used as a break crop in arable rotations increased the acreage grown of oilseed rape. It was not until the 1970's, that the expansion of crop production has given oilseed rape a major agricultural role (Table

1.1) due to the UK's membership of the European Economic Community (EEC), which instituted price incentives under the Common Agricultural Policy (C.A.P.), and the explosion of world commodity prices.

The effect of the C.A.P. of the E.E.C. was to stabilise the market for oilseed rape in Europe, giving farmers a middle-term to long-term approach on oilseed growing with the knowledge of what price his crop was likely to fetch and not prone to wild fluctuations caused by world market conditions.

**Table 1.1 Production of rapeseed (in '000 tonnes)**

| | 1961 | 1970 | 1980 | 1990 |
|---|---|---|---|---|
| United Kingdom | 2 | 8 | 90 | 1258 |
| World | 4048 | 6600 | 11000 | 26000 |

Source: Oilseed Rape Book

Initially oilseed rape cultivars mainly consisted of spring varieties because they fitted ideally as a break crop with the established cereal growing pattern. There was a gradual swing from spring to winter cereal crops, which gave higher yields and were less susceptible to the unpredictability of summer weather conditions as spring varieties. In order to follow the cereal growing patterns, growers started switching from spring to winter oilseed cultivars, therefore winter oilseed rape is now the principal type grown in the U.K. The different varieties are also classed in relation to their erucic oil and glucosinolate content, which are the main criteria in determining the final product for consumer usage. High erucic oil content is suitable for lubricants,

whilst low erucic oil content is suitable for human consumption. The requirement for animal rapeseed meal is a low glucosinolate content as a high content can cause metabolic disorders in animals. Advances in scientific research and breeding strategies has improved crop performance and end product quality to maintain the market demand and economic advantages to the grower.

### 1.2.2 Environmental Impact - Feral OSR and Potential Transgenic Cross Contamination

The expansion of oilseed rape as a crop has led to the increase of volunteer or feral plants due to high seed losses prior and post-harvest. Seeds can persist in the soil through induced ecodormancy for many years giving rise to substantial feral populations (Pekrun *et al.*, 1997). These feral populations establish themselves not only on agricultural land but also roadside verges through seed spillage during post-harvest transportation, waste tips and semi-natural habitats (Raybould & Gray, 1993).

Temporal models of feral oilseed rape seedbank dynamics showed persistence of over five years (Squire *et al.*, 1997b), this was confirmed by genetic fingerprinting of feral populations which revealed that certain cultivars are capable of persisting for five years and intercultivar hybridisation within the feral population occurs (Charters, 1996).

Studies of seedbank persistence on wild species x crop hybrids have shown that this persistence would increase, especially if the wild plants were the female parent, due to the increased dormancy and effective germination cueing responses (Linder & Schmitt, 1994).

The heterozygosity level of oilseed rape has an average of 30% with variations, due to different environmental conditions, ranging from 12% to 47% (Becker *et al.*, 1992). In field experiments, workers have reported spontaneous hybridization between

oilseed rape and weedy species (Eber *et al.*, 1994; Jorgensen & Anderson, 1994; Rogers & Parkes, 1995). These plants of crop x wild hybrid produce self-fertile seeds that can thereafter backcross with one of the original parents (Brown & Brown, 1996; Mikkelsen *et al*, 1996).

Method of pollen transfer is either wind or insect mediated with the former being judged the most important in pollen dispersal (Langridge & Goodman, 1982). Research into pollen emission, dispersal and possible hybridization with plants of related species has given a wide range of results. Small plot trials by McCartney & Lacey (1991) reported a 90% decrease in pollen density over 10 m, whilst Timmons *et al.* (1995) reported a 90% decrease at 360 m. from large commercial fields. Hokanson et al. (1997) found a 90% reduction in gene movement at a distance of 3 m from its source.

The critical factors determining pollen spread of oilseed rape are: local weather condition, plot size, and the stage of development of the pollen plume from the crop (Crawford *et al.*, 1996). The magnitude of hybridization with other related species is subject to the duration of pollen release, synchrony of flowering and the amount of pollen produced by each cultivar.

Using a molecular biology technique termed somatic hybridization foreign DNA can now be inserted into crop species, that could not be achieved by normal breeding, through protoplast fusion, micro-injection and gene vector systems (Jones & Cassells, 1995). This appeals to the plant breeder in that the desired foreign DNA confers new properties upon the crop. This method also cuts the time cycle from hybridization to commercial release in breeding programmes from 12 to four years (Dunwell, 1996).

Genetically engineered plants are mainly designed for crop protection as fungal, bacterial and viral diseases, in addition to pests and weeds, cause major losses to agricultural production systems. Conventional disease control involves a substantial outlay in cost and time to ensure a healthy crop therefore enhancing the plant's genetic self-defence mechanisms it will reduce pesticide and herbicide usage (Harms, 1992). The major problem for oilseed rape growers is having an effective weed control as infestation of the crop can cause a 10% reduction in yield and also lowers the end quality of the product. Herbicide application causes damage to the crop in addition to the primary objective of eradicating the weeds. Herbicide resistant genotypes are increasingly becoming the most common form of genetically engineered trait, mainly for glyphosate, imidazolinone, sulfonylurea and glufosinate herbicides (Miki *et al*, 1990)

The majority of agricultural crops are being bred for distinct qualities to meet the demands for specific consumer products, hence the large diversity of inter-cultivar variation. Oilseed rape is very amenable to transformation, mainly through *Agrobacterium* infection of tissue explants, and has a high regenerative efficiency. Designer crops are being produced to enhance the quality of the various fatty acids according to the end product required. The development of novel oil crops, where new fatty acids from uncultivated species are being investigated for their commercial uses, are increasing and it will only be a matter of time before the desired genetic material carrying the synthesis of the fatty acid can be successfully transferred from the wild plant to an oilseed rape cultivar (Murphy, 1995).

The environmental concern in using transgenic crops is the potential escape of genes to the environment that could lead to undesirable change. This could occur via genetic space through pollen transfer to other crops and wild species resulting in

hybridization (Till-Bottraud *et al*, 1992; Arias & Rieseberg, 1994; Schmitt & Linder, 1994; Lanbo *et al*, 1996). The seeds from transgenic species can form feral seedbanks that can persist over a long period (Raybould & Gray, 1993).

All transgenic crops must go through regulatory systems in order to test the risk of genetic escape and potential ecological damage, but there are a number of issues that must always be considered: Genetically modified organisms (GMO's) are generally tested as small-scale field trials; their behaviour under large scale releases is uncertain and might increase the probability of establishment; there is the lack of long-term genetic predictability of the GMO and the unknown ecological predictability if transgenic escape occurs (Williamson, 1992).

In 1986 only one application for field release of transgenic crop plants had been made to governments in the Organisation for European Co-operation and Development (OECD) countries, by 1992 this number had risen substantially to 399 and included 175 applications for oilseed rape (Table 1.2), the highest figure for any single crop (Sumida, 1996). The only area that genetically modified oilseed rape has been tested for the potential of escaped transgenes is in seed survivorship and dormancy. In comparisons with conventional oilseed rape it was found that the ecological performance was similar and there was no increase in potential invasiveness of the GMO's (Crawley *et al.*, 1993; Linder & Schmitt, 1995,)

**Table 1.2 Annual approvals for field release of transgenic crops granted in OECD countries**

| | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 |
|---|---|---|---|---|---|---|
| Alfalfa | 0 | 1 | 7 | 4 | 3 | 6 |
| Brocolli | 0 | 0 | 0 | 7 | 3 | 4 |
| Cantaloupe | 0 | 0 | 0 | 0 | 0 | 1 |
| Cauliflower | 0 | 0 | 0 | 0 | 1 | 1 |
| Chicory | 0 | 0 | 0 | 1 | 1 | 3 |
| Corn | 0 | 0 | 0 | 2 | 23 | 40 |
| Cotton | 0 | 0 | 5 | 9 | 9 | 14 |
| Flax | 0 | 1 | 5 | 6 | 13 | 24 |
| Melon | 0 | 0 | 0 | 1 | 1 | 2 |
| Oilseed Rape | 0 | 5 | 15 | 41 | 54 | 175 |
| Poplar | 0 | 1 | 2 | 2 | 1 | 0 |
| Potato | 2 | 8 | 12 | 21 | 38 | 52 |
| Rice | 0 | 0 | 0 | 2 | 1 | 1 |
| Soybean | 0 | 0 | 4 | 5 | 5 | 26 |
| Sugarbeet | 0 | 0 | 1 | 8 | 8 | 10 |
| Sunflower | 0 | 0 | 0 | 1 | 1 | 0 |
| Tobacco | 3 | 7 | 9 | 20 | 19 | 13 |
| Tomato | 3 | 12 | 7 | 14 | 18 | 18 |

Source: Sumida (1996)

### 1.2.3 Oilseed Rape - Breeding & Cultivar Stability

Oilseed rape is classified into two distinct groups, annual and biennial, according to their vernalization requirement for inducing flowering. Another

differentiation is made in relation to their geographical location, i.e. European and Asiatic cultivars, in which both annual and biennial types are found.

The genetic backgrounds of European winter and summer oilseed rape indicate distinct accessions mainly due to intra-group breeding with very little inter-crossing between the two sets (Diers & Osbourne, 1994). The Asian cultivars of winter and summer types make up a third genetically distinct group due to extensive hybridization between *B. napus* L. and *B. campestris* in Japanese development programmes (Erickson *et al.*, 1983).

Traditional plant breeding methods for oilseed rape consist of using in-bred lines to produce a progeny that is backcrossed with the original parents to acquire the dominance in the specified traits (Davey, 1941). The seeds are selfed through to the sixth generation going through the process of selection each time for the traits required producing a uniform and stable population. Compared to a heterogeneous variety, an inbred line flowered and ripened more uniformly, but was more susceptible to disease.

The elite breeding method (Lööf, 1975) consists of producing seed that was very heterogeneous, in which progeny from selected plants were open pollinated in order to have a wide genetic base as possible. Emphasis was put on varietal heterogeneity, because of the loss of yield from inbreeding depression and heterogeneity buffered the crop from disease and weather conditions.

The development of new cultivars with improved qualities has catapulted oilseed rape into a major commodity on the world agricultural market in the past two decades. The first breakthrough occurred with the first double low variety that had a low erucic acid content of less than 5% and a low glucosinolate content of less than 30 μmol/g. Currently there is great interest in new breeding strategies to increase yield and improve quality. One such method is heterosis where a single intervarietal hybid

cross produces the desired $F_1$ progeny that has a yield of 40-60% in excess of its parents (Sernyk & Stefansson, 1983). The success of this system depends on three criteria: an acceptable level of heterosis, sufficient pollen transfers to the seed parents; and an effective means of pollination control (Grant & Beversdorf, 1985). Pollination control is achieved using three methods: cytoplasmic male sterility (CMS); genic male sterility (GMS); and self-incompatability.

Another method in breeding new cultivars of oilseed rape is by resynthesizing the cultivar by crossing *B. oleracea* with *B. campestris*, its ancestral parents. The rationale is to increase desirable parental traits, *B. oleracea* and *B. campestris* having a greater genetic variation than *B. napus* L. (Chen & Heneen, 1989).

Cultivar stability and uniformity will depend on the breeding method; Charters *et al.* (1996b) found differing degrees of genetic variation existing in inter- and intra-cultivar comparisons of oilseed rape. Whilst there are EEC legislative requirements in operation pertaining to distinctiveness, uniformity and stability to protect plant breeders rights, which means new cultivars having less genetic variability (Thompson, 1983), this does not prevent new phenotypic and genotypic cultivars being formed through hybridization of mixed cultivars in feral seedbanks.

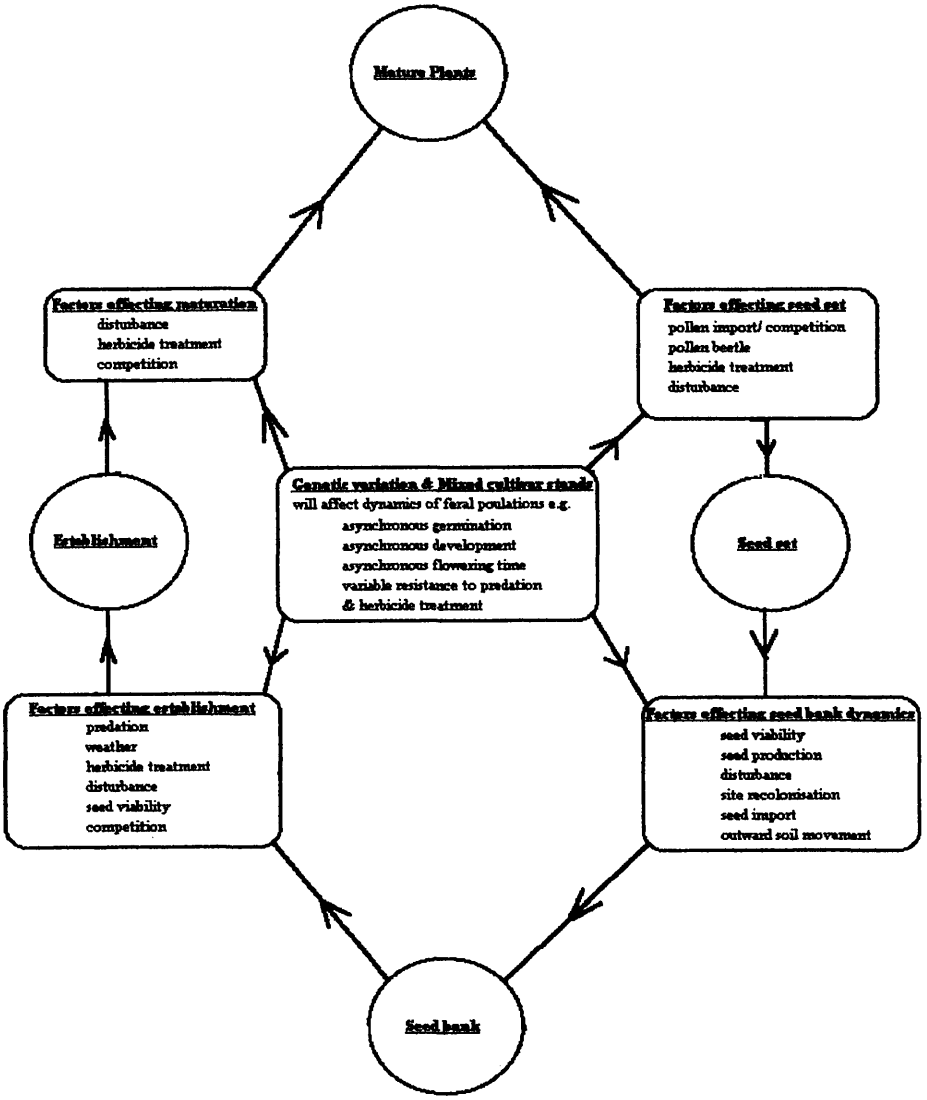
### 1.2.4 Dynamics of the Feral Oilseed Rape Seedbank

The majority of feral seed banks outside agricultural fields are established through seed spillage from mechanised transportation and the outward movement of soil containing seeds. The seedbank in its first year has to survive predation, plant competition, herbicide treatment and environmental conditions in order to firmly establish itself. In conditions favourable for germination prior to the onset of winter,

plants may be produced in a relatively short period from the time of seed transfer. The seeds that do not germinate pre-winter are induced into secondary dormancy and emerge in the following spring or summer subject to seed viability. The regeneration of the seedbank is dependant on the fecundity of the plants to maintain population numbers.

The dynamics of the seedbank population are affected by intra-cultivar genetic variation and mixed cultivar stands in response to environmental conditions. Inter plant gene transfer is at its maximum when asynchronous germination, development and flowering time occurs. Temperature is the principal environmental component that dictates the commencement and duration of an individual seed's lifecycle. A process of natural selection occurs in seedbank populations in response to varying environment conditions, which confers a genetic adaption within its available genepool to attain higher fitness (Brown & Venable, 1986). This could occur where the environmental conditions favours germinators of a specific genotype for a series of years whereby there is a natural increase in the population pool towards that genotype. Seeds that lie dormant for a series of years due to unfavourable conditions can contribute to the seedling population of later generations (Sagar & Mortimer, 1976).

Dormancy and seed germination characteristics of the seedbank will vary if hybridisation occurs between differing cultivars or between cultivated and wild types (Landbo & Jørgensen, 1997). In effect, the variation in annual weather cycles leads to an ever changing population of seed number, genotype and phenotype (Beckstead *et al*, 1996). The interactions between temperature, dormancy, germination and genotype must be examined to understand the complexities of the dynamics of seedbank populations (Figure1.1).




Source : Charters (1996)

**Figure 1.1 Feral population dynamics**

### 1.2.5 Phenotypic Variation & Genotypic Identification

Germination studies on seeds in response to temperature have related time and temperature to phenotype (Garcia-Huidobro *et al.*, 1982; Acharya *et al.*, 1983; Washitani, 1987; Nykiforuk & Johnson-Flanagan, 1994). The limitation to these studies has been whether a phenotype at a given temperature is the same at another temperature, i.e. is an early germinator at a low temperature also an early germinator at a high temperature? This is because morphological identification of individual seeds within a population could not be determined and once a seed has germinated it cannot be re-used again in subsequent experiments.

Germination characteristics in response to environmental conditions are defined genetically, so the broader the genetic variation within a species would produce a broader germination response, this has shown in rate-temperature germination experiments for oilseed rape (Marshall & Squire, 1996; Squire *et al*, 1997a).

If a specific germination response was observed at different temperatures for a known genotype then a full germination profile can be developed for the different oilseed rape cultivars. These profiles in turn could lead to the development of mathematical models to predict various outcomes of germination, plant development and flowering in response to environmental conditions. This information would be very valuable in assessing dynamics feral seedbank populations and the risks of using genetically modified cultivars.

Oilseed rape plants are difficult to identify on the basis of morphology. Measurements and analysis systems of their chemical compositions e.g. seed oil fatty acid profiles were not adequate or sensitive enough to enable accurate discrimination between cultivars. With new cultivars being constantly developed by breeders, having

only slight but important physiological differences from their parental material it was important to have a satisfactory identification system for oilseed rape.

Molecular biology in the 1980's was taking great strides forward, especially in the area of genetic mapping. In 1987 the method of restriction fragment length polymorphism (RFLP) was being used to construct a detailed genetic map for *Lactuca sativa* L. (Landry *et al.*, 1987) and was later used for *Brassica napus* L. (Landry *et al.*, 1991). The disadvantage of this system was the large amounts of probes/DNA required and the protracted procedures involved to secure sufficient genetic information for cultivar indentification.

A method that was simpler to use and increased the genetic information by producing more polymorphic banding came to the fore in 1991 using random amplified polymorphic DNA (RAPD) markers for *Brassica oleracea* (Hu & Quiros, 1991). The RAPD method consists of using synthesised oligodeoxynucleotide primers 9 or 10 nucleotides in length, between 50% and 70% G+C composition with no palindromic sequences (Williams *et al.*, 1990). This method was used to discriminate between 23 cultivars of *Brassica napus* L. using six primers producing 23 polymorphic bands (Mailer *et al.*, 1994).

It was reported in 1987 that the human genome contained repetitive DNA sequences such as variable number tandem repeat (VNTR) loci (Nakamura *et al.*, 1987) and were referred to as minisatellites. Other research revealed high levels of polymorphism exist in dinucleotide tandem repeat sequences where $(CA/GT)_n$ occurred up to 50,000 times with n varying between 10 and 60 (Weber & May, 1989). These DNA sequences were termed simple sequence repeats (SSR) or microsatellites. This research was extended to plants (Akkaya *et al.*, 1992; Lagercrantz *et al.*, 1993) using PCR primers that amplified the inter-SSR sequences. Polymorphism was found

in all *Brassica* species using $(GT)_n$ and $(CT)_n$ microsatellites. Further investigation of *Brassica napus* L. revealed an abundance of simple sequence repeats that were highly polymorphic in character with the most common being GA-, CA-, and GATA- (Poulsen *et al.*, 1993; Kresovich *et al.*, 1995).

The SSR method cannot only discriminate between cultivars, but also within cultivars (Charters *et al.*, 1996a). Charters found that 20 cultivars of *Brassica napus* L. could be identified with only two 5'-anchored SSR primers. Investigations of intra-cultivar polymorphism using a minimum of 10 individuals revealed substantial variation with some cultivars exhibiting a high degree of polymorphism between individuals whilst others showed no polymorphism.

Cluster analysis of banding profiles for *Brassica napus* L. revealed distinct groupings in three areas: winter/spring varieties, high/low glucosinolate content, and geographic origin of cultivar.

A comparison between SSR, amplified fragment length polymorphism (AFLP), RAPD and RFLP markers for genetic analysis has revealed that SSR's have the highest expected heterozygosity and that the information generated is the most compatible with the requirements of plant breeding and population genetics (Powell *et al.*, 1996).

### 1.2.6 Seed Dormancy

The survival of some types of plant species depends on its successful reproduction and dispersal of its progeny. Most plants disperse their progeny in the form of seeds and to be successful await favourable conditions for growth. The variation in most climatic cycles for different geographical areas requires a period of inactivity for the seed, where growth is completely arrested in order to survive a period

of adverse environmental conditions (Osborne, 1981). This period is known as "Dormancy" and is the temporary suspension of visible growth of any plant structure containing a meristem (Lang, 1987).

The range of dormancy induction and break varies with plant species and habitat and is controlled through the integration of regulatory plant and environment interactions (Lang, 1994). The dormancy period in a given seed lot normally has a broad distribution therefore variation in seed germination within a population will occur which ensures the maximisation of a plant species' survival in a broad environmental spectrum as possible (Milberg & Andersson, 1997).

There are many aspects and types of dormancy occurring in stages of plant growth, e.g. innate, induced and enforced dormancy (Lang *et al.*, 1987). Innate dormancy, sometimes referred to as primary dormancy, is the state where a seed will not germinate when all the environmental criteria have been met for commencement of germination. Induced or secondary dormancy occurs when a seed that is able to germinate, goes into a state of dormancy after being subjected to a specific adverse environmental condition, and can no longer germinate under favourable growth conditions. Enforced dormancy is the state where a seed fails to germinate due to adverse environmental conditions. The seeds of *Brassica napus* L. in a natural habitat will not exhibit a primary dormancy when the seeds are shed onto the ground and are highly germinable, but they can have an induced secondary dormancy due to unfavourable environmental conditions that prevents germination until dormancy loss occurs (Pekrun *et al.*, 1997).

Dormancy mechanisms tend to be lost in the breeding of annual crops due to the intentional selection for uniformity in germination (Adler *et al.*, 1993). The agricultural practice of maintaining the viability of seeds through post-harvest dry

after-ripening, followed by prolonged storage in cool conditions, removes seed dormancy. Therefore germination will be triggered on receipt of beneficial environmental conditions (Ellis & Roberts, 1981). In many species the environmental triggers for the induction and loss of dormancy is "sensed" in the seed coat and is therefore determined solely by the genetics of the maternal parent (Naylor, 1983; Garbutt & Witcombe, 1986; Qi *et al.*, 1993). Even when the loss of innate dormancy in seeds occurs, germination-inducing factors are still required before germination can commence. Even if germination-inducing factors are present a single germination-preventing factor can induce seeds into secondary dormancy (Roberts, 1981).

Temperature plays a key role in removing dormancy in a seed before germination can commence (Monte del & Tarquis, 1997;Vleeshouwers, 1998). The seeds of certain species need a period of low temperatures (stratification) before germination can commence, and this has an important ecological significance. This chilling effect causes a loss of primary dormancy, at the same time inducing a secondary dormancy, so that germination will occur on transfer to a higher temperature (Roberts, 1981). Some plant species e.g. winter varieties of oilseed rape, also require a period of low temperatures to occur for flower initiation to commence, this requirement is called vernalization. Seeds that germinate in late summer to early autumn and subsequently develop vegetative rosettes require a seasonal timing mechanism for reproductive development to be initiated the following spring. The most common vernalization requirements are a photoperiod of 12 hours combined with temperatures of 0 -10°C (Metzger, 1996).

Other species need alternating temperatures of diurnal variation to lose dormancy. The different factors responsible for dormancy loss in alternating temperature regimes include: the upper and lower temperature values, the time spent at

the maximum and minimum temperatures, the number of diurnal cycles, the rate of warming and cooling (Roberts, 1988). The disadvantages of conducting laboratory experiments in simulation of diurnal temperatures, are that the complex and irregular temperature cycles that occur naturally cannot be matched artificially therefore reducing the understanding of the dynamics between alternating temperature fluctuations and dormancy.

### 1.2.7 Seed Germination: Influence of Temperature

Temperature plays a key role in both seed dormancy and seed germination. In seed germination it is a major germination-inducing factor and also controls the rate of germination. The majority of seeds usually have a broad temperature range in which germination occurs to allow for environmental fluctuations during its growth period. The temperature at which seeds germinate most rapidly is referred to as the optimum temperature whilst the minimum temperature at which a seed of any given species will germinate is referred to as the base temperature. Thermal time, a parameter used in germination rate/temperature calculations, is the accumulated temperature in degrees per day above the base temperature. Oilseed rape has a broad temperature range for germination with an optimum temperature of around 20°C (Figure 1.2.) at which both the rate of germination and the proportion which germinate are maximal (Acharya *et al.*, 1983; Kondra *et al.*, 1983). At either side of this optimum the rate and percentage of germination decline as the temperature differential increases. It has been found that there is intra- and inter-cultivar variation in germination characteristics in oilseed rape with base temperatures varying from 0.4°C to 5°C (Morrison *et al.*, 1989; Marshall & Squire, 1996; Vigil *et al.*, 1997).

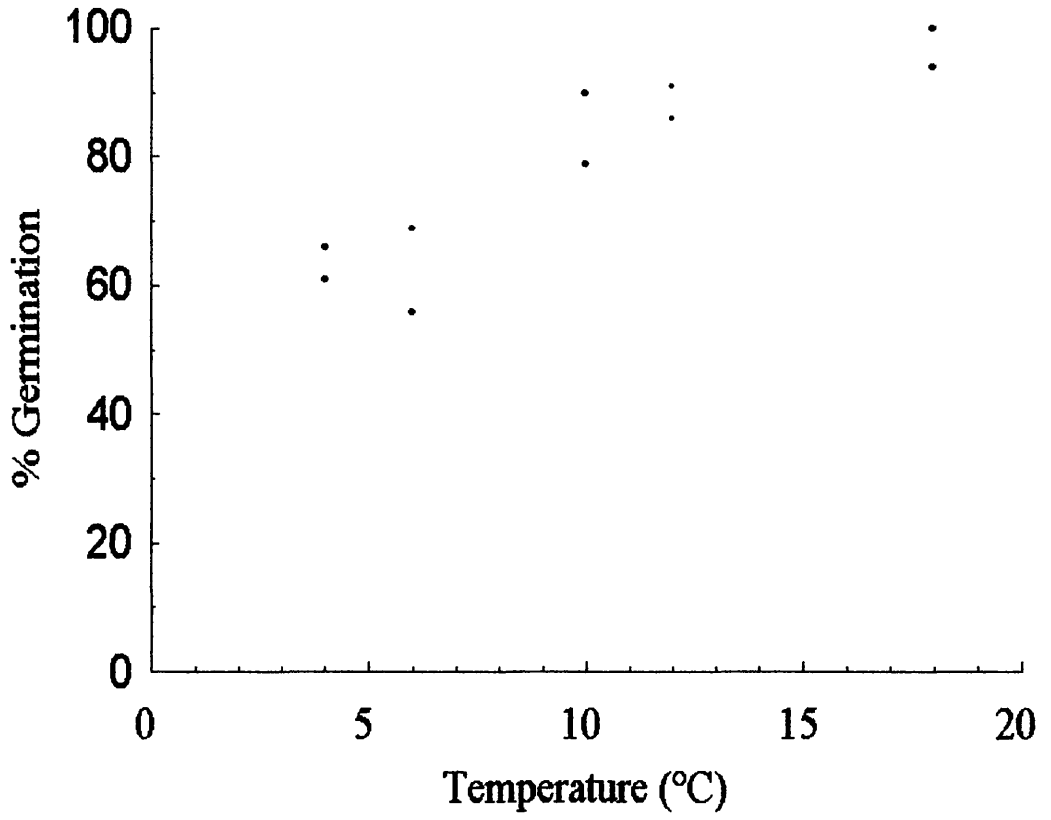



**Figure 1.2** **Total germination of oilseed rape cv. Martina in response to temperature**

Within a given seed population in most crop plants kept at a constant temperature there is a linear relationship of germination rate and temperature $T$ between the base $T_b$ and optimum $T_o$.

The relationship between rate of germination and time can be quantified by

$$1/t = (T-T_b)/\theta$$

Where $1/t$ is the rate of germination; $\theta$ is thermal time; $T$ is temperature; and $T_b$ is the base temperature. These linear relationships between temperature and rate of germination have been found in the majority of the main crop plants. However it has been recently found that cultivars of oilseed rape exhibit non-linearity, especially at low temperature. This non-linearity results in a faster germination time for the earliest fraction in a seed lot.

Some cultivars (e.g. Rocket) show a germination pattern over time that is consistent over a broad range of temperatures (4 - 20°C). We refer to these as 'uniform' cultivars. In contrast, other cultivars (eg. Martina) show a range of germination patterns as temperature is altered. We refer to these as 'variable' cultivars. A proportion of the seeds do not germinate (the 'non-germinating' trait) at constant, cooler temperatures. This proportion increases as temperature is reduced, and is viable, germinating when temperature is increased. Another apparent feature of these 'variable' cultivars is that the spread of germination times (e.g. time between the 10th percentile and 90th percentile) at any given temperature is greater than the 'uniform' cultivars studied so far.

Marshall & Squire (1996) in seed germination experiments at constant temperatures applied mathematical growth curves called generalised logistic curves, to cumulative germination at each monitored temperature and accounted for 98% of the variation in seed germination data sets. The purpose of using generalised logistic curves was to provide an adequate description or an acceptable fit to the data.

$$y = y_{max}/(1+t(e^{-b(x-m)})^{1/t})$$

where $y$ is percentage germination at time $x$; $y_{max}$ is the final percentage germination; $t$ defines the shape of the response ($t > 0$); $b$ the steepness of the response; and $m$ its location on the time axis.

There are two special cases of the generalised logistic function:

(1) where these general logistic curves approached the lower boundary of the shape parameter ($t = 0$), the curves were refitted using the Gompertz function.

$$y = y_{max} e^{-e^{-b(x-m)}}$$

(2) when $t = 1$, the logistic function is used and the curve is asymmetrical about the point of inflection.

$$y = y_{max} / (1 + (e^{-b(x-m)}))$$

From these time to germination curves, the time of germination for the 10th, 20th, 50th, 80th and 90th percentile can be found (Figure 1.3).

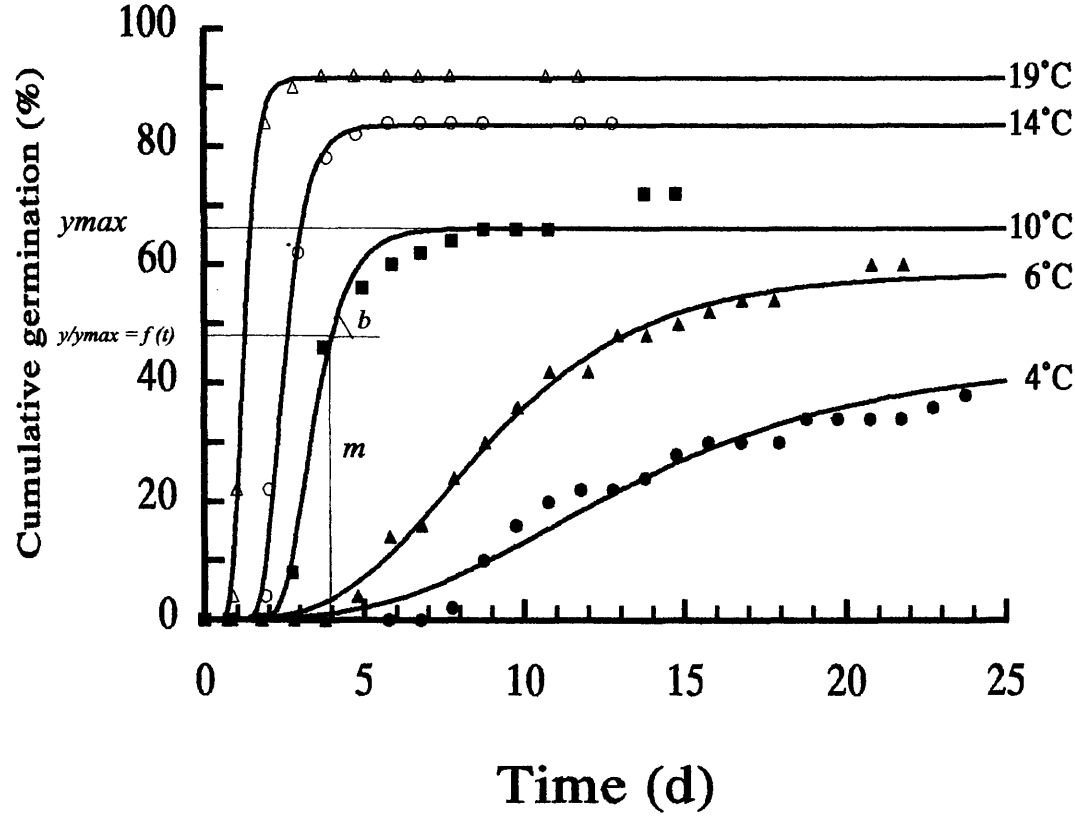



**Figure 1.3 Time to germination response of oilseed rape cv. Martina**

The non-linearity of these germination responses were then examined between germination rate and temperature and curves fitted for the 10th, 20th, 50th, 80th and

90th percentiles (Figure 1.4). In order to determine how the percentiles differed in the shape of the germination curve and the temperature at which the rate of germination extrapolated to zero, an exponential model function was fitted.

$$1/t = a_i(b^{(T-T_b)}-1)$$

Where $a_i$ is a scaling parameter that is the multiplier of the basic response function; the subscript $i$ defines the value of $a$ and varies with the 10th, 20th, 50th, 80th and 90th percentiles of germination; and $b$ is a shape parameter that is common to all percentiles (Squire *et al.*, 1997a).

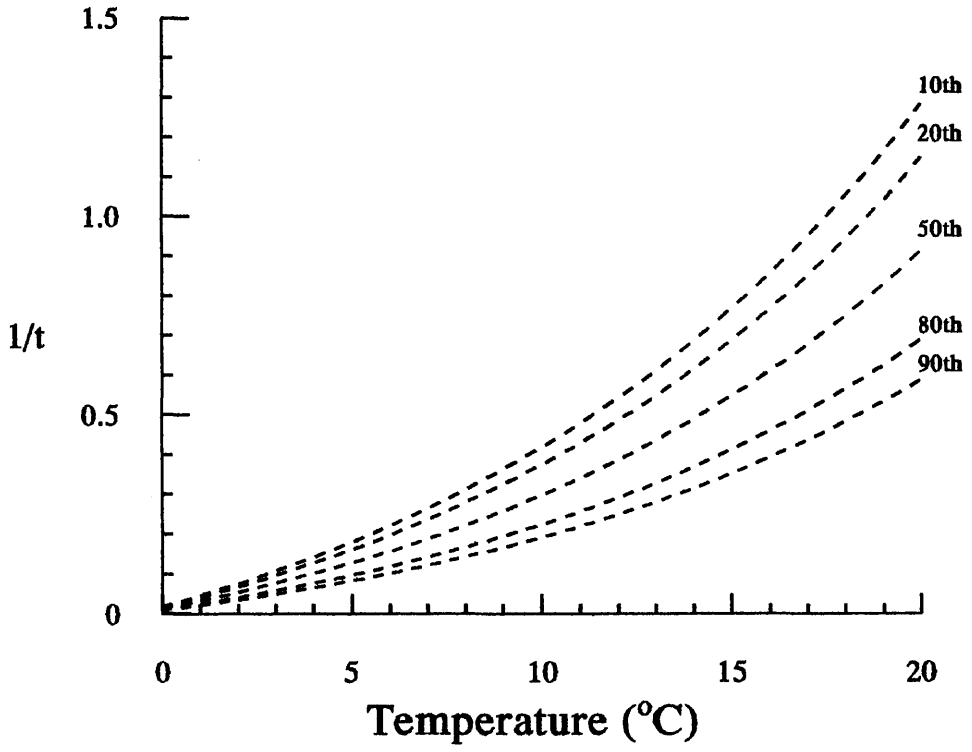



**Figure 1.4 Fitted curves of 1/time on temperature for oilseed rape cv. Martina**

### 1.2.8 Seed Germination: Influence of Water & Osmotic Potential

Germination starts when the seed starts to imbibe water and is complete when the radicle emerges from the seed coat. During the first phase, imbibition continues with the rate of water uptake depending on the permeability of the seed coat, the osmotic potential of the available water and the biological composition of the seed. The components of the seed act in a hydrophilic colloidal fashion and therefore swell with the uptake of water (Edwards, 1968).

The major components that swell in seeds are the storage proteins. During the rest phase hydrolytic enzymes break down the storage proteins into nitrogenous substances in order to be utilised by the seed. The main storage proteins in *Brassica napus* are napin and cruciferin that comprises 20% and 60% respectively of the total protein content of mature seeds and are rapidly degraded within 3 to 4 days of germination. (Hoglund *et al.*, 1992)

Germination rate decreases with decreasing water potential until a critical threshold is reached under which seeds will not germinate. The main factors governing the effect of water potential on germination in field conditions are the soil moisture levels and size of soil aggregates (Hadas, 1977).

The variation in germination response to water potential is far less than the germination response to temperature (Witcombe & Whittington, 1971; Dunlap, 1988; Livingston & de Jong, 1990).

### 1.2.9 Seed Germination: Influence of Light

The influence of light on seed germination can be divided into 4 main categories: seeds that only germinate a) in the dark, b) in the light, c) with a diurnal

light variation as in photoperiodism and d) seeds that are indifferent to light conditions.

The effect of light on the germination of the majority of wild species of seeds depends on their natural habitat and environmental life cycle (Baskin and Baskin, 1996; Schütz, 1997). Certain species at or near the soil surface use their phytochrome system in their seed coat as a light-sensing device, in the red/far-red wavelength, which contributes to triggering germination (Wesson & Waring, 1968; Hazebroek & Metzger, 1990).

Whilst wild species of *Brassica rapa* showed a distinct response to light levels (Adler *et al.*, 1993), within cultivated species there is very little evidence that light influences germination. There is one notable exception of a light/dormancy interaction affecting germination, Pekrun *et al.* (1997) found that oilseed rape seeds that were induced into ecodormancy by stressful environmental conditions and kept in conditions of darkness showed a light sensitivity in which exposure to light stimulated dormancy loss.

## Chapter 2

## MATERIALS AND METHODS

### 2.1 *Project Overview*

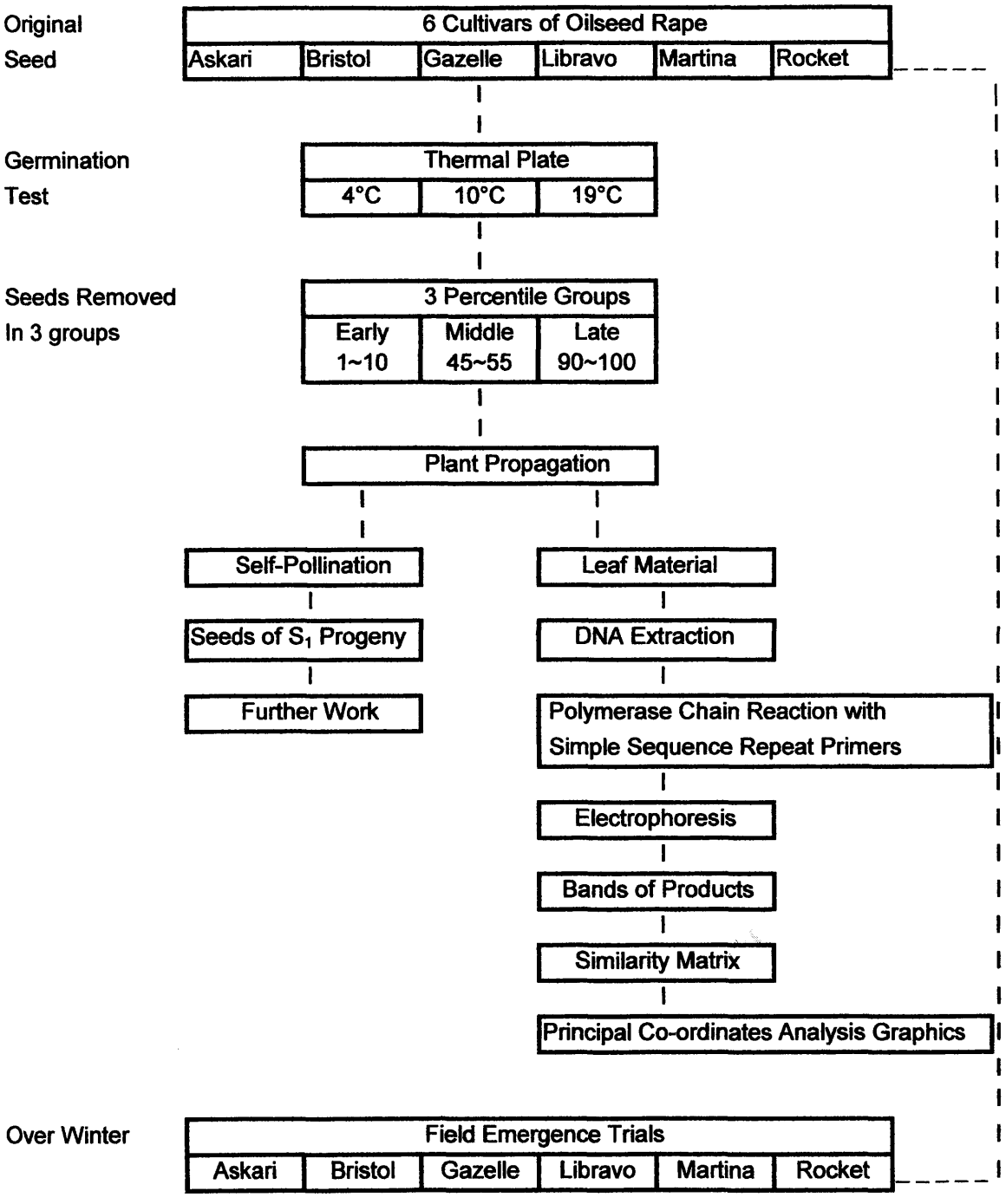



**Figure 2.1 Experimental overview**

## 2.2 SEED GERMINATION

### 2.2.1 Cultivar selection

Six cultivars of the winter type variety were selected based on the degree of genetic polymorphism exhibited in previous studies (Charters *et al.*, 1996a) (Table 2.1) with the objective of having a contrasting range of genetic diversity within cultivars.

**Table 2.1 Oilseed rape cultivars used in experiment**

| *Cultivar* | *Type* | **No of variable bands* |
|---|---|---|
| Askari | Double high | 3 |
| Bristol | Double low | 0 |
| Gazelle | Double low | 0 |
| Libravo | Double low | 10 |
| Martina | Double high | 9 |
| Rocket | Double low | 1 |

* No of variable bands using primer SCRI No 1420

### 2.2.2 Temperature selection

The temperatures selected for germination were chosen to produce slow, average and fast rates of germination. Scientific literature has indicated that 19°C is close to, but still below, the optimum germination temperature, and 4°C is just above the base temperature for most oilseed rape cultivars. It has been reported that 10°C is near the optimum temperature for genotypic separation by phenotype (Acharya *et al.*, 1983; Nykiforuk & Johnson-Flanagan, 1994), i.e. cultivars that differ both in their genetic base and germination rates will exhibit the maximum separation in time for germination between seedlots.

### 2.2.3 Instrumentation – laboratory germination test

The temperature incubation apparatus used for seed germination was a two-way thermo gradient plate model 91e manufactured by Grant Instruments (Cambridge) Ltd., U.K. Thermocouples positioned on the thermal plate (Figure 2.2) were constructed of fine wire type 'K' chromal/alumel, (0.127 mm diameter), via miniature thermocouple connectors to solid wire type 'K' chromal/alumel, (0.5 mm diameter). All components were manufactured by TC Ltd.

The thermocouples were connected to a Campbell 21X model ESX data logger (Appendix 1) that recorded temperatures every 1 minute, from which the hourly average, maximum and minimum temperatures were calculated and stored.

The processing of the temperature data was accomplished using SPLIT, a Campbell Scientific software package. The average temperatures for rows 2, 5, and 10, where the seeds were placed on the thermal plate, was calculated assuming there was a uniform temperature gradient between each thermocouple.

### 2.2.4 Germination and phenotypic selection

The thermal plate was overlaid with blotting paper linked to side wells filled with water, to keep the germination surface moist. A grid of 14 x 14 boxes, each 25cm$^2$, was placed on top of the blotting paper and covered with glass plates to minimise external influences on temperature fluctuations and loss of moisture.

Seedlots from the six cultivars were positioned in their selected boxes on the thermal plate, shown in (Figure 2.2), in three rows, each row representing nominal temperatures of 4°C, 10°C and 19°C respectively. A seedlot consisted of 50 seeds; there were two replicates of each cultivar per temperature.

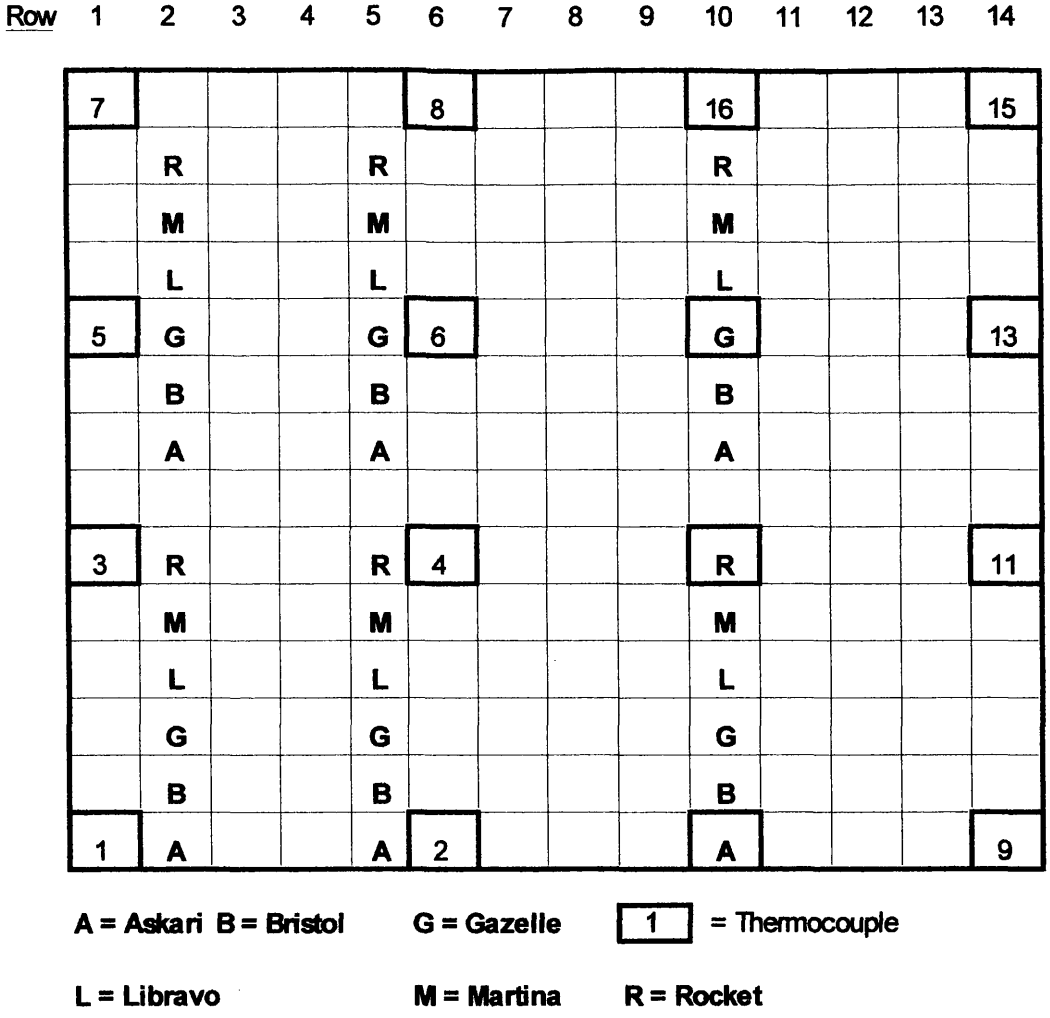



**Figure 2.2 Position of Seedlots on Thermal Plate 1996**

Starting dates for germination were staggered, coolest first and hottest last, so that seed from all treatments tended to achieve maximum germination at approximately the same time. This was done to allow the simultaneous application of alternating temperature changes required for non-germinators in all the temperature groups, after the constant temperature regime.

Germination tests were started on 16 April, 17 April & 6 May 1996. Observations were made on a daily basis, except in the initial stages at 10°C and 19°C, when observations were made every three hours and every hour respectively. Germination of a seed was deemed to occur when a radical, 1 mm in length, was visible.

The constant temperatures were maintained until all germination had ceased. By June 1996 all seed at 19°C had germinated, but a proportion of seed at 10°C and a greater proportion of seed at 4°C had not. These seed are referred to as non-germinators. These proportions remained constant, i.e. no more seeds germinated and on the 17 June the remaining non-germinated seeds from the 4°C and 10°C groups were moved to new locations at warmer temperatures (Table 2.2). Further increases of temperature were made and finally on 3 July a regime of alternating temperature was applied. This final regime resulted in the germination of all the remaining viable seeds.

**Table 2.2 Increases of temperature applied to non-germinating fraction of seed 1996**

| Date | Initial temperature | New temperature |
|---|---|---|
| 17 June | 4°C | 10°C |
| 17 June | 10°C | 16°C |
| 24 June | 4°C | 16°C |
| 24 June | 10°C | 22°C |
| 28 June | 4°C | 21°C |
| 28 June | 10°C | 25°C |
| 3 July | 4°C | Alternating |
| 3 July | 10°C | Alternating |

## 2.3 SEED PROGENY

### 2.3.1 Plant propagation

Seeds that germinated on the thermal plate in the first, middle and final viable 10 per cent were transferred in small containers to an unheated glasshouse for propagation; seeds out with these selected percentiles were discarded.

At the stage when 4 leaves had emerged (dicotyledons plus two true leaves) after four weeks growth, a sample of leaf tissue was taken from the youngest leaf of each plant, placed on ice and stored at a temperature of -80°C. Approximately two weeks later, the plants were placed in a vernalization cabinet (5°C, 12/12 h day/night) for eight weeks.

### 2.3.2 Self-pollination and seed extraction

Successive batches of vernalized plants were placed in 15 cm diameter pots filled with John Innes Compost, and transferred to an unheated glasshouse for further growth during the period August until October 1996. Pollination bags were fitted on plants when their first flower appeared. Agitation of the pollination bags on a daily basis ensured that self-pollination occurred. All the plants in the cold glasshouse were transferred to a heated glasshouse (Table 2.3) with sodium lighting in December 1997 to continue growing, flowering and seed ripening through the winter period.

**Table 2.3 Average glasshouse temperatures during period of flowering**

| Month | Unheated Glasshouse | Heated Glasshouse |
|---|---|---|
| September 1996 | 20.1°C | |
| October 1996 | 14.5°C | |
| November 1996 | 14.5°C | |
| December 1996 | | 20.7°C |
| January 1997 | | 22.4°C |
| February 1997 | | 24.2°C |
| March 1997 | | 19.2°C |
| April 1997 | | 23.8°C |
| May 1997 | | 23.6°C |
| June 1997 | | 23.2°C |

The plants were harvested through April until May 1997 and stored in a drying area for three months. The seed progeny from every individual plant was extracted by hand, weighed and collected separately for use in further work.

## 2.4 DNA EXTRACTION

### 2.4.1 DNA extraction method 1

The first method of extraction was according to Hu & Quiros (1991) with modifications as used by Charters (1996).

*Reagents*

| | |
|---|---|
| Liquid Nitrogen | |
| Extraction Buffer (pH 8.0) | 100 mM Tris-HCl<br>50 mM EDTA-$Na_2$<br>500 mM NaCl, 1.25% (w/v) SDS |
| Potassium acetate 5 M | 4°C |
| Iso-propanol | ice cold |
| TE buffer (pH 8.0) | 10 mM Tris-HCl<br>1 mM EDTA-$Na_2$ |
| Ribonuclease A | 10 mg/ml (Sigma) |
| Sodium Acetate 3 M | room temperature |
| Ethanol | ice cold |
| Chloroform: IAA | 24:1 |

*Protocol*

- Weigh 150 mg of leaf tissue from a stored sample kept at -80°C.
- Grind to a fine powder using liquid nitrogen with a mortar and pestle.
- Transfer to a microfuge tube, add 500 µl of extraction buffer and store on ice.
- Incubate in a water-bath at 65°C for 15 minutes.
- Add 200 µl of potassium acetate and refrigerate at -20°C for 10 minutes.

- Centrifuge at 14,000g for 5 minutes and transfer supernatant to a new tube.
- Add 600 µl of Iso-propanol to supernatant and invert gently to precipitate DNA.
- Centrifuge at 14,000g for 10 seconds to pellet DNA.
- Discard Iso-propanol and air-dry the pellet at room temperature.
- Add 250 µl of TE buffer, 5 µl of Rnase A and incubate in a water-bath at 65°C for 15 minutes to dissolve DNA.
- Add 25 µl of Sodium acetate and 500 µl of Ethanol, incubate at -20°C for 20 minutes.
- Centrifuge at 14,000g, discard supernatant and re-suspend in 250 µl of TE buffer.
- Add 500 µl of Chloroform/IAA and shake on an orbital rotator for 15 minutes.
- Transfer the upper aqueous layer to a new tube.
- Repeat the Iso-propanol precipitation and centrifugation steps.
- Re-suspend DNA pellet in 250 µl of TE buffer.
- Store at 4°C (short-term) prior to DNA quantification.

### 2.4.2 DNA extraction method 2

The second extraction method was according to Sul and Korban (1996) with modifications that reduced the time taken considerably whilst maintaining the high purity and quality required.

*Reagents*

| | |
|---|---|
| Liquid Nitrogen | |
| Extraction Buffer (pH 8.0) | 100 mM Tris-HCl<br>50 mM EDTA-$Na_2$<br>500 mM NaCl, 1.25% (w/v) SDS |
| Chloroform: IAA | 24:1 |
| Iso-propanol | ice cold |
| Ethanol 70% | room temperature |
| TE buffer (pH 8.0) | 10 mM Tris-HCl<br>1 mM EDTA-$Na_2$ |

Ribonuclease A 10 mg/ml (Sigma)

*Protocol*

- Weigh 150 mg of leaf tissue from a stored sample kept at -80°C.
- Grind to a fine powder using liquid nitrogen with a mortar and pestle.
- Transfer to a microfuge tube and add 700 µl of extraction buffer.
- Pipette solution gently several times to mix sample thoroughly and store on ice.
- Incubate in a water-bath at 65°C for 5 minutes and swirl frequently.
- Add 700 µl of Chloroform/IAA and mix to form an emulsion.
- Centrifuge at 14,000g for 2 to 3 seconds.
- Transfer the top aqueous layer of supernatant (approx 250 µl) to a new tube.
- Add 1 ml of Iso-propanol to supernatant and invert gently to precipitate DNA.
- Centrifuge at 14,000g for 10 seconds to pellet DNA.
- Discard Iso-propanol and wash twice with 1 ml of 70% Ethanol.
- Air-dry the pellet at room temperature.
- Add 250 µl of TE buffer and incubate in a water-bath at 65°C for 10 minutes to dissolve DNA.
- Add 5 µl of Rnase A and store at 4°C (short-term) prior to DNA quantification.

### 2.4.3 DNA quantification

DNA concentrations and purity were determined using an Ultrospec III spectrophotometer at wavelengths of 260 and 280 nm respectively.

*Protocol*

- Set the spectrophotometer to function 1 (DNA concentration and purity).
- Use 980 µl of TE buffer for the standard of zero absorbance.
- Add 20 µl of DNA to standard
- Take readings of DNA concentration and purity

Calculations were made based on the DNA concentration to determine the amount of sterile distilled water to add to an aliquot of 25 µl of extracted DNA to give a concentration of 10 µg DNA/µl in each PCR reaction mixture.

## 2.5 DNA ANALYSIS USING PCR WITH SSR PRIMERS

### 2.5.1 PCR protocol

*Reagents*

| | | |
|---|---|---|
| $H_2O$ | 11.8 µl | Sterile distilled Water |
| 1.5 mM $MgCl_2$ buffer | 2.0 µl | Boehringer Mannheim |
| 2 mM dNTP's* | 2.0 µl | Boehringer Mannheim |
| 3 µM SSR primer | 2.0 µl | SCRI (Chemistry Dept.) MWG Biotech |
| Taq DNA polymerase | 0.2 µl | Boehringer Mannheim |

* Equal amounts of ATP, CTP, GTP & TTP

*Protocol*

- All the reaction components were combined in a 2 ml microfuge tube made up of the quantity stated above multiplied by the number of samples + control.
- Aliquots of an 18 µl reaction mixture were transferred to 0.2 ml microfuge tubes.
- 2 µl of the DNA template was added to each of the tubes and then 2 µl of sterile distilled water added to the controls.
- PCR amplifications were carried out using a Techne (Cambridge) Genius Thermocycler incorporating a heated lid using a three-stage programme:

♦ A 5 minute delay to warm-up heated lid.

♦ 30 cycles of 1 minute at 94°C, 2 minutes at 55°C, 30 seconds at 72°C.

♦ 1 cycle extension of 5 minutes at 72°C with finish holding temperature of 4°C.

PCR amplification was carried out using two simple sequence primers, SCRI No. 1420 (BDB-CACACACACA) & SCRI No. 1425 (BDV-CAGCAGCAGCAGCAG). Each primer consisted of a repeat sequence preceded by a 5' anchor that has a 3 variable base position. The anchors were designated the code letters accordingly B = C, G or T; D = A, G or T; V = A, C or G.

### 2.5.2 Gel electrophoresis of PCR products

The PCR amplification products were separated on a Multiphor II cooled (10°C) flatbed system using pre-cast polyacrylamide gels (Cleangel 48S, Pharmacia Biotech).

*Reagents*

| | |
|---|---|
| Gel rehydration buffer | 75 mM Tris-acetate |
| Electrode buffer | 200 mM Tris base (Sigma) |
| | 200 mM Tricine (Sigma) |
| | 0.55% (w/v) SDS |
| Loading buffer | 0.02% (w/v) Bromophenol blue |
| | 5 M Urea |
| 123 Bp DNA ladder | Sigma |
| Lambda DNA EcoR 1 Hind III digest | Sigma |

*Protocol*

- Switch on recirculation water pump with temperature set at 10°C.
- Rehydrate the polyacrylamide gel for 1 hr in 200 ml buffer.
- Dry the gel surface with filter paper.
- Apply a small quantity of distilled water onto plate surface and position gel.
- Moisten 2 sets (11 strips of 23 cm x 4 cm, Whatman No1 Chromatography paper) of filter wicks with 21 ml of electrode buffer.
- Position each wick 1 cm in from edge of gel.
- Add 3 µl of loading buffer to each sample of PCR product.
- Load 7 µl of sample per well, load 5 µl of each DNA ladder in the end wells.
- Position electrodes and connect to power supply (Consort)
- Run electrophoresis controlled by a three stage programme:

  (1) 30 minutes at 200 Vmax, 13 mAmax, 7 Wmax.

  (2) 1 hour at 380 Vmax, 20 mAmax, 13 Wmax.

  (3) 1 hour 20 minutes at 450 Vmax, 20 mAmax, 13 Wmax.

### 2.5.3 Detection of bands

Band detection was obtained using the plus one silver staining method (Pharmacia Biotech) to ensure standardisation of gel development.

*Reagents*

| | |
|---|---|
| Fixing | Benzene sulphonic acid 3% w/v |
| | Ethanol 24% v/v |
| Silver impregnation | Silver nitrate 1% w/v |
| | Benzene sulphonic acid 0.35% w/v |
| Washing | Distilled water |
| Development | Sodium carbonate 12.5% w/v |
| | Formaldehyde 37% w/v |
| | Sodium thiosulphate 2% w/v |
| Stopping & Preserving | Acetic acid 5% w/v |
| | Sodium acetate 25% w/v |
| | Glycerol 50% v/v |

*Protocol*

- 30 minutes fixing in 125 ml of reagent.
- 30 minutes silver staining in 125 ml of reagent.
- 1 minute washing in 150 ml of distilled water.
- 6 minutes developing in 125 ml of reagent.
- 30 minutes stopping & preserving in 125 ml of reagent.

The complete staining procedure was carried out in stainless steel trays on a 3D rocking platform (Stuart Scientific).

### 2.5.4 Analysis of banding

Band positions from individual plant profiles were visually identified using a 2x magnifier (Jencons) and recorded as present or absent. The number and

position of bands scored varied with each cultivar. Approximately 20 bands per primer for each cultivar were used for analysis.

Data analysis was conducted using the Genstat V (Numerical Algorithms Group Ltd) software package (McConway *et al*, 1999).

The Nei & Li (Nei & Li, 1979) similarity matching method was used,

$$\text{where similarity} = \frac{d}{b+c+d}$$

The similarity between two individuals can be estimated from a comparison of their respective band maps; this method is the most appropriate index for comparing individuals within a cultivar using a dominant marker system such as SSR's.

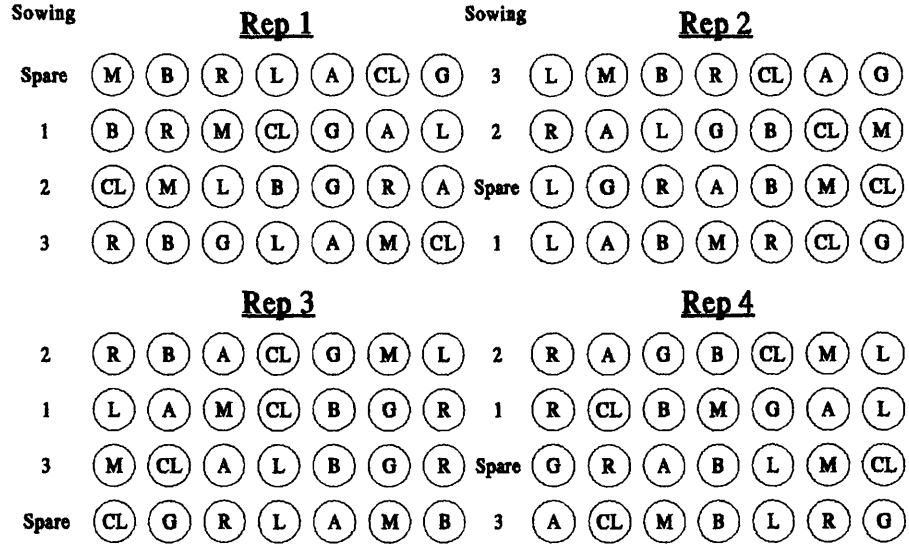
These individuals can be represented in a space where the separation distances are proportional to the genetic distance in the triangular matrix. This is achieved by using a standard mathematical procedure called Principal Co-ordinate (PCO) analysis where the genetic variation can be described within the first three axes. The PCO vectors generated, are similar in principle to the way inter-town distances are represented at the back of road maps. These PCO vectors were inserted into a Stanford Graphics 3.0b (Visual Numerics Inc.) software package to produce a 3-dimensional map that represents the genetic space of the intra-cultivar population.

## 2.6 OVERWINTERING FIELD TRIALS

### 2.6.1 Field preparation

In September 1997, an area approximately 22 m x 11 m was ploughed and rotovated in Laboratory field for over-wintering germination trials. All weeds were removed and the surface overlaid with Phormisol (LBS Horticulture), white in colour, to reduce the light intensity but allow water to permeate in order to deter further weed growth. Holes were cut in the tarpaulin, pots 30 cm in diameter were buried in the soil with their rims at two cm above the surface and filled with sieved soil. The sowings were arranged as split-plots, for ease of access, randomised with each replicate. The fourth sowing locations were not required (Figure 2.3).

FIELD PLAN




A = Askari B = Bristol CL = Control G = Gazelle

L = Libravo M = Martina R = Rocket

**Figure 2.3 Field experiment layout**

### 2.6.2 Environmental monitoring equipment

All pots were fitted with thermocouples solid type 'K' chromal/alumel, 0.5 mm diameter (TC Ltd) connected via multiplexers to two Campbell 21X data loggers (Appendix 2) powered by solar panels. The soil temperatures were monitored every 10 minutes and recorded the average hourly temperature, the daily maximum and minimum temperatures. The control pots were fitted with TDR probes (Trace Ltd) to monitor the soil moisture.

### 2.6.3 Sowing and emergence of cultivars

Each sowing consisted of seedlots containing 100 seeds for each of the six cultivars and a control pot that was not sown. The seeds were uniformly spread over the surface and then covered with a layer of sieved soil, 2 cm in depth. Slug pellets were spread around the perimeter of each pot after sowing to prevent any infestation. Three sowings were made; the fourth sowing was not required because a sustained period of cold weather occurred in January 1998 creating the dormancy conditions required.

**Table 2.4 Dates of sowing field experiment**

| Date | Sowing |
|---|---|
| 31/10/97 | 1 |
| 17/11/97 | 2 |
| 21/01/98 | 3 |

Emergence was deemed to occur when the cotlyedon leaves were open. Observations of emergence were made and recorded on a daily basis. After emergence, each seedling from the first and last fifteen percent of emergence was transferred to seed trays and placed in a heated glasshouse with sodium lighting to encourage growth. Leaf samples for DNA analysis were taken at an early stage and placed in cold storage. In March 1998, the young plants were placed in 15 cm pots and transferred to an unheated glasshouse for natural vernalization and future self-pollination for use in further studies.

## Chapter 3

# PHENOTYPIC VARIATION OF GERMINATION TRAITS

## 3.1 Introduction

It is known that phenotypic variation in germination exists between and within oilseed rape cultivars (Marshall & Squire, 1996). This variation exists not only in rate of germination, but also in seed response to low temperature (Squire *et al*, 1997a) through the fraction entering secondary dormancy, the depth of secondary dormancy and the different signals needed for dormancy break (Pekrun *et al*, 1997). The purpose of the germination test (Figure 2.1) was to examine these responses and to quantify the variation by means of rate-temperature parameters in six different oilseed rape cultivars, knew to have varying degrees of genetic polymorphism (Table 2.1).

## 3.2 Constant temperature

Given that from previous experiments there was systematic variation in temperature across the plate, the seedlots were divided into two replicates split at right angles to the axis of variation.

**Table 3.1 Seedlot temperatures calculated from thermocouple measurements**

| Nominal Temperature | Replicate | Mean | Abs. Maximum | Abs.Minimum |
|---|---|---|---|---|
| 4°C | 1 | 4.0°C | 4.5°C | 3.3°C |
| 4°C | 2 | 3.2°C | 4.5°C | 2.8°C |
| 10°C | 1 | 10.1°C | 10.6°C | 9.4°C |
| 10°C | 2 | 9.4°C | 10.2°C | 9.1°C |
| 19°C | 1 | 19.0°C | 20.4°C | 18.4°C |
| 19°C | 2 | 18.6°C | 19.0°C | 18.4°C |

The mean, absolute maximum and absolute minimum temperatures for each seedlot were calculated from recorded thermocouple measurements (Table 3.1). Seeds in replicate 2 experienced slightly lower temperatures than in replicate 1.

All cultivars achieved a high final percentage at 19°C (Table 3.2); Libravo had the lowest germination figure at 19°C and appeared more prone to fungal infection on its seed coat than the other cultivars and this may have been the cause of its reduced viability.

Bristol had a large fraction of seed (mean 59%) that exhibited non- germination at 10°C and this fraction increased (mean 78%) further at 4°C. Martina and Libravo showed a small reduction in germination at 10°C. Like Bristol, Martina and Libravo showed a further reduction in germination at 4°C. Askari, although showing no effects at 10°C did show a small reduction at 4°C. The results for Libravo are inconclusive in view of fungal infections. In most cultivars, maximum germination was lower in replicate 2 at 4°C.

**Table 3.2 Final percentage germination attained at constant temperature**

| | Nominal Temperature | | | | | |
|---|---|---|---|---|---|---|
| | 4°C | | 10°C | | 19°C | |
| rep | 1 | 2 | 1 | 2 | 1 | 2 |
| cv. | | | | | | |
| Askari | 78 | 64 | 88 | 96 | 92 | 88 |
| Bristol | 30 | 14 | 40 | 42 | 98 | 100 |
| Gazelle | 86 | 80 | 100 | 94 | 98 | 100 |
| Libravo | 74 | 50 | 82 | 86 | 76 | 84 |
| Martina | 52 | 52 | 78 | 82 | 90 | 100 |
| Rocket | 92 | 82 | 100 | 100 | 88 | 98 |

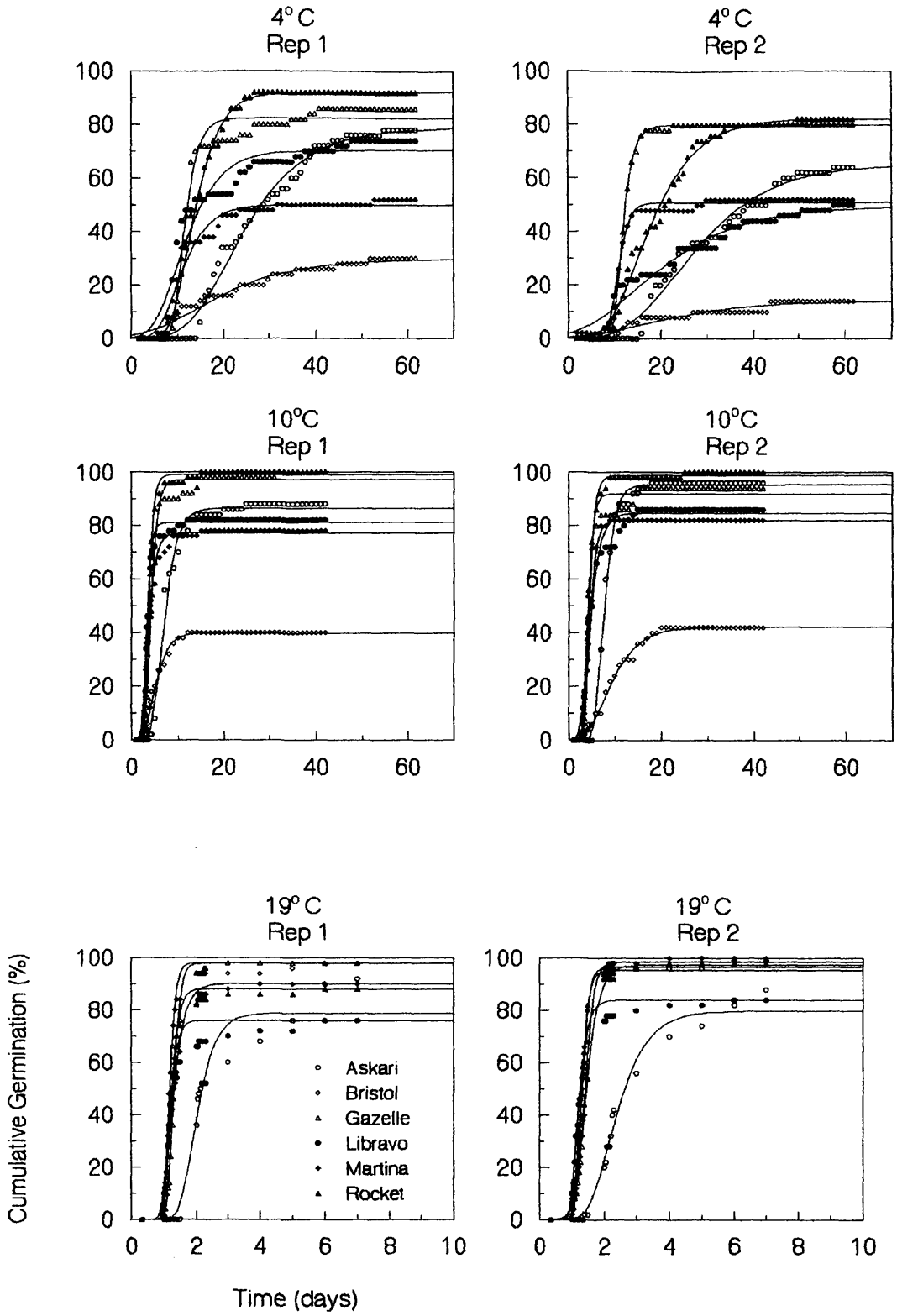



**Figure 3.1 Time to germination data and fitted curves at 4°C, 10°C and 19°C**

Time to germination curves at constant temperatures of 4°C, 10°C and 19°C (Fig. 3.1) confirm the greater fraction of non-germination with decreasing temperatures. Askari showed a poor initial time to germination response (a late germinator), but exhibited a comparable germination rate at all three temperatures. Bristol had slower initial time to germination and germination rates at 4°C and 10°C, but was one of the fastest cultivars at 19°C. The other cultivars of Gazelle, Libravo, Martina and Rocket had similar curves for 10°C and 19°C, with distinct differences being apparent at 4°C.

Cumulative germination curves were fitted to germination data sets for each temperature using Genstat V. The curves used were generalised logistic, logistic and gompertz functions:

(1) Glogistic: $y = y_{max} / (1+t(e^{-b(x-m)})^{1/t})$ (see Fig 1.3, page 22)

(2) Logistic: $y = y_{max} / (1+(e^{-b(x-m)}))$

(3) Gompertz: $y = y_{max}\, e^{-e^{-b(x-m)}}$

where $y$ = percentage germination at time $x$, $y_{max}$ is the final percentage germination, $t$ defines the shape of the response, $b$ the steepness of the response and $m$ its location on the time axis. Curves 2 and 3 are special cases of the generalised logistic: logistic ($t$ = 1) and gompertz ($t$ = 0).

The model tried to fit the generalised logistic function, but where it failed to converge due to $t$ approaching 0, the gompertz function was then fitted. The $t$ values were always less than 1 and therefore the logistic curve was not required. In any event, the prime concern was the closeness of fit, by which criteria the glogistic will always be as good as and generally better than the logistic. Variance accounted for was over 99% in 23 out of the 36 data sets (Table 3.3).

**Table 3.3 Generalised logistic, logistic and gompertz functions for time to germination curves**

| Temperature | cv. | $b$ | $m$ | $y_{max}$ | $t$ | type | % accounted for |
|---|---|---|---|---|---|---|---|
| 4°C Rep 1 | Askari | 0.108 | 21.0 | 78.9 | 0 | gompertz | 99.0 |
| | Bristol | 0.086 | 14.1 | 29.8 | 0 | gompertz | 96.6 |
| | Gazelle | 0.503 | 10.7 | 82.3 | 0.011 | glogistic | 98.0 |
| | Libravo | 0.193 | 9.6 | 70.3 | 0 | gompertz | 95.1 |
| | Martina | 0.253 | 9.4 | 49.9 | 0 | gompertz | 97.5 |
| | Rocket | 0.259 | 12.1 | 92.1 | 0 | gompertz | 99.8 |
| 4°C Rep 2 | Askari | 0.092 | 23.2 | 65.1 | 0 | gompertz | 98.3 |
| | Bristol | 0.081 | 15.9 | 14.0 | 0 | gompertz | 91.3 |
| | Gazelle | 0.632 | 11.2 | 79.9 | 0.011 | glogistic | 100.0 |
| | Libravo | 0.083 | 14.5 | 49.5 | 0 | gompertz | 96.4 |
| | Martina | 0.710 | 10.6 | 51.0 | 0.524 | glogistic | 99.0 |
| | Rocket | 0.148 | 15.1 | 82.1 | 0 | gompertz | 99.3 |
| 10°C Rep 1 | Askari | 0.533 | 6.2 | 86.5 | 0 | gompertz | 99.4 |
| | Bristol | 0.551 | 4.5 | 39.9 | 0 | gompertz | 99.1 |
| | Gazelle | 1.084 | 3.9 | 97.2 | 0.011 | glogistic | 99.1 |
| | Libravo | 1.658 | 3.1 | 81.2 | 0.011 | glogistic | 99.0 |
| | Martina | 0.968 | 3.2 | 77.2 | 0.011 | glogistic | 98.7 |
| | Rocket | 1.429 | 3.5 | 99.2 | 0.011 | glogistic | 99.7 |
| 10°C Rep 2 | Askari | 0.664 | 7.1 | 95.4 | 0 | gompertz | 99.9 |
| | Bristol | 0.244 | 7.8 | 42.3 | 0 | gompertz | 99.5 |
| | Gazelle | 1.285 | 3.8 | 92.0 | 0.011 | glogistic | 98.7 |
| | Libravo | 0.644 | 3.9 | 84.7 | 0 | gompertz | 98.2 |
| | Martina | 1.062 | 4.1 | 82.0 | 0.011 | glogistic | 99.9 |
| | Rocket | 1.172 | 4.0 | 98.8 | 0.011 | glogistic | 99.8 |
| 19°C Rep 1 | Askari | 2.537 | 1.9 | 78.8 | 0 | gompertz | 96.8 |
| | Bristol | 8.789 | 1.1 | 94.5 | 0 | gompertz | 99.6 |
| | Gazelle | 8.190 | 1.3 | 95.8 | 0.349 | glogistic | 99.6 |
| | Libravo | 6.672 | 1.1 | 69.5 | 0 | gompertz | 98.2 |
| | Martina | 4.042 | 1.2 | 88.1 | 0 | gompertz | 99.6 |
| | Rocket | 6.804 | 1.2 | 85.0 | 0.057 | glogistic | 99.6 |
| 19°C Rep 2 | Askari | 1.569 | 2.2 | 79.8 | 0 | gompertz | 98.6 |
| | Bristol | 7.797 | 1.3 | 95.3 | 0.239 | glogistic | 99.7 |
| | Gazelle | 3.753 | 1.3 | 97.5 | 0 | gompertz | 99.7 |
| | Libravo | 5.425 | 1.1 | 79.6 | 0 | gompertz | 99.3 |
| | Martina | 3.941 | 1.3 | 98.5 | 0 | gompertz | 99.4 |
| | Rocket | 8.128 | 1.3 | 96.6 | 0.840 | glogistic | 99.9 |

note: Gompertz is the limiting case of the generalised logistic function when t = 0. The gompertz estimates the three remaining parameters $b$, $m$ and $y_{max}$.

There was a predominance of gompertz type curves, 23 out of 36 curves, with the rest being of the generalised logistic type. Nine of the generalised logistic curves had a very small $t$ value of 0.011 suggesting that they were close to the gompertz function. A gompertz type curve, where $t$ describing the shape of the response is approaching infinity, occurs in a seed population where the acceleration of germination rate reaches its maximum in the early percentiles and reduces thereafter. The timepoint $m$, which is the point of inflection where the slope of parameter $b$ is at its maximum, indicates when there is a reduction in germination rate. Libravo and Martina had the smallest $m$ of approximately 9.5 days and 3.1 days at 4°C and 10°C respectively, showing a faster rate of germination of the early percentiles in comparison to the other cultivars. At 19°C, there was no apparent difference in $m$ between the cultivars.

The variation in the time course of germination for the six cultivars was further explored by examining the relationship between temperature and the germination rate $1/t$ (Squire *et al.*, 1997a), for different percentiles. Two exponential models were fitted using the FITNONLINEAR directive in Genstat V:

(1) $y_1 = a_i(b^{(T-T_b)}-1)$

(2) $y_2 = a_i(b_i^{(T-T_b)}-1)$

where $y$ = germination rate; $T_b$ is the base temperature; $a_i$ is a scaling parameter that is the multiplier of the basic response function; the subscript $i$ indicates that the value of $a$ varies with the percentile of germination under investigation; and $b$ is a shape parameter that is common to all percentiles in model 1 but varies with percentile in model 2.

Both models were run and the results compared. Model 1 was to be the first choice; model 2 was only used if it gave a significant improvement of an increase in the overall fraction accounted for (Table 3.4).

**Table 3.4 Parameter estimates and their standard errors in parenthesis, overall fraction of variation accounted for ($r^2$) in either model 1 and/or 2 as appropriate**

| | Askari | Bristol | Gazelle | Libravo | Martina | Rocket |
|---|---|---|---|---|---|---|
| $T_b$ | -1.4 | -0.9 | -2.0 | -0.6 | -1.8 | 0.2 |
| | (1.54) | (3.78) | (1.86) | (1.35) | (2.28) | (0.60) |
| *b* | 1.107 | * | 1.099 | 1.078 | 1.096 | 1.085 |
| | (0.013) | * | (0.014) | (0.015) | (0.018) | (0.008) |
| *b10* | * | 1.154 | * | * | * | * |
| | * | (0.028) | * | * | * | * |
| *b20* | * | 1.195 | * | * | * | * |
| | * | (0.027) | * | * | * | * |
| *b50* | * | 1.244 | * | * | * | * |
| | * | (0.169) | * | * | * | * |
| *b80* | * | 1.257 | * | * | * | * |
| | * | (0.191) | * | * | * | * |
| *b90* | * | 1.250 | * | * | * | * |
| | * | (0.210) | * | * | * | * |
| *a10* | 0.090 | 0.059 | 0.149 | 0.306 | 0.174 | 0.270 |
| | (0.039) | (0.061) | (0.073) | (0.143) | (0.108) | (0.061) |
| *a20* | 0.080 | 0.027 | 0.139 | 0.281 | 0.157 | 0.252 |
| | (0.034) | (0.029) | (0.068) | (0.131) | (0.097) | (0.057) |
| *a50* | 0.061 | 0.011 | 0.120 | 0.233 | 0.130 | 0.218 |
| | (0.026) | (0.032) | (0.059) | (0.109) | (0.080) | (0.049) |
| *a80* | 0.046 | 0.008 | 0.102 | 0.128 | 0.104 | 0.182 |
| | (0.019) | (0.025) | (0.050) | (0.059) | (0.064) | (0.041) |
| *a90* | 0.044 | 0.008 | 0.091 | * | 0.103 | 0.173 |
| | (0.019) | (0.028) | (0.045) | * | (0.064) | (0.039) |
| $r^2$ | 0.995 | 0.993 | 0.990 | 0.991 | 0.987 | 0.996 |
| *s.e.obs* | (0.015) | (0.03) | (0.029) | (0.033) | (0.036) | (0.02) |

Model 1 gave the best fit for all the cultivars with the exception of Bristol, consistent with previous findings for Martina (Squire *et al,* 1997a). There was no significant difference in $T_b$ or *b* between the cultivars, most within two s.e.s of each other indicating that parameter *a* is responsible for differences in germination rate between cultivars. The ranges and values of parameter *a* indicated which cultivars are the early germinators and how uniform is the germination rate. According to the model, Libravo and Rocket should be the quickest to initial germination followed by Martina and Gazelle. Askari, Bristol, Gazelle and Martina show a range of *a* values

from the 10th to the 90th percentile of between approximately 0.05 to 0.07 difference whilst Libravo has 0.17 and Rocket 0.1 difference.

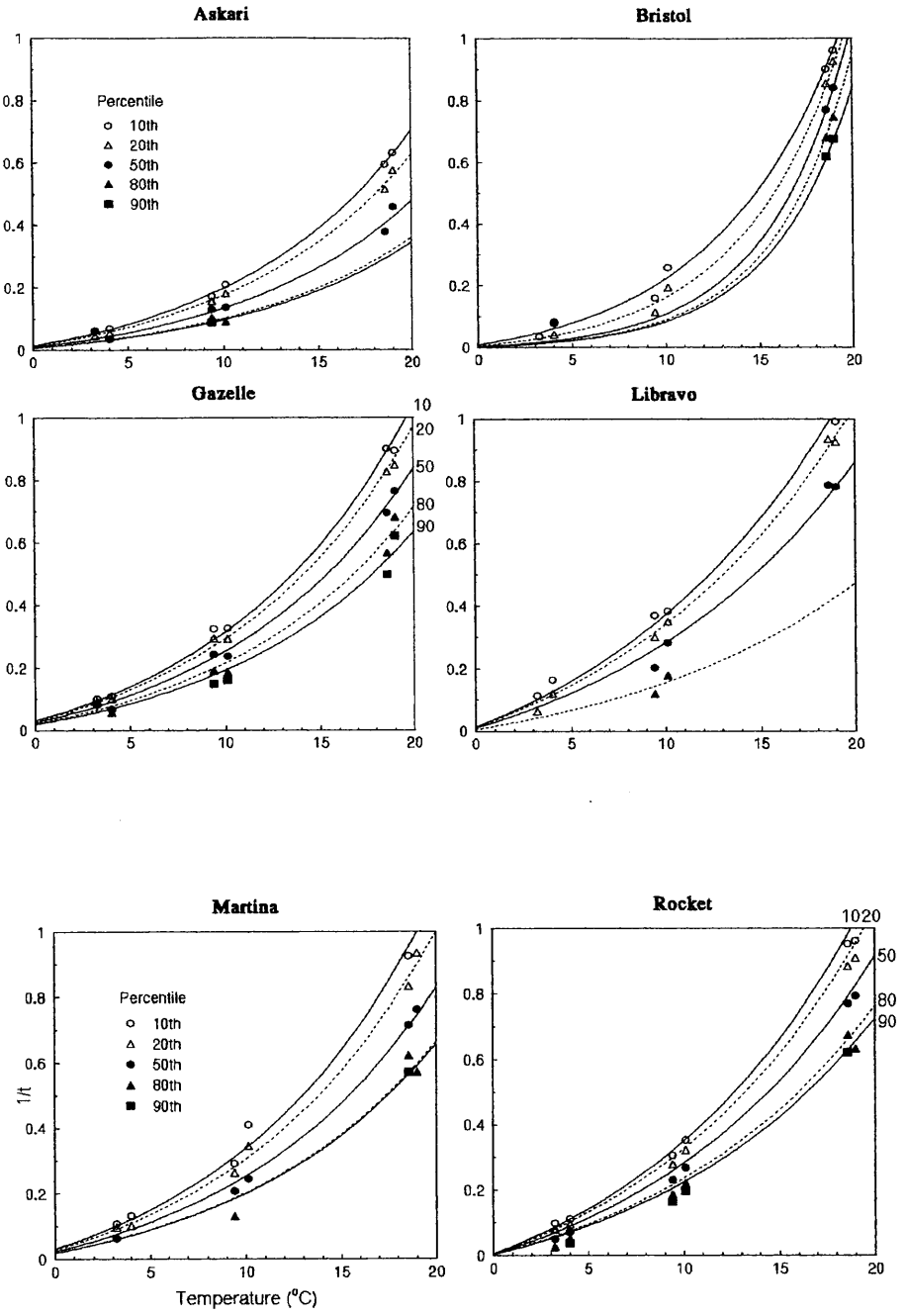



**Figure 3.2 Rate of germination in relation to temperature for all percentile groups**

The data and curves of best fit (Figure 3.2) provide visual confirmation that parameter a was the main discriminator of within-cultivar differences, that the slope of the curves was different in Bristol, and that non-germination at 19°C distorted the curve for the 80th percentile in Libravo.

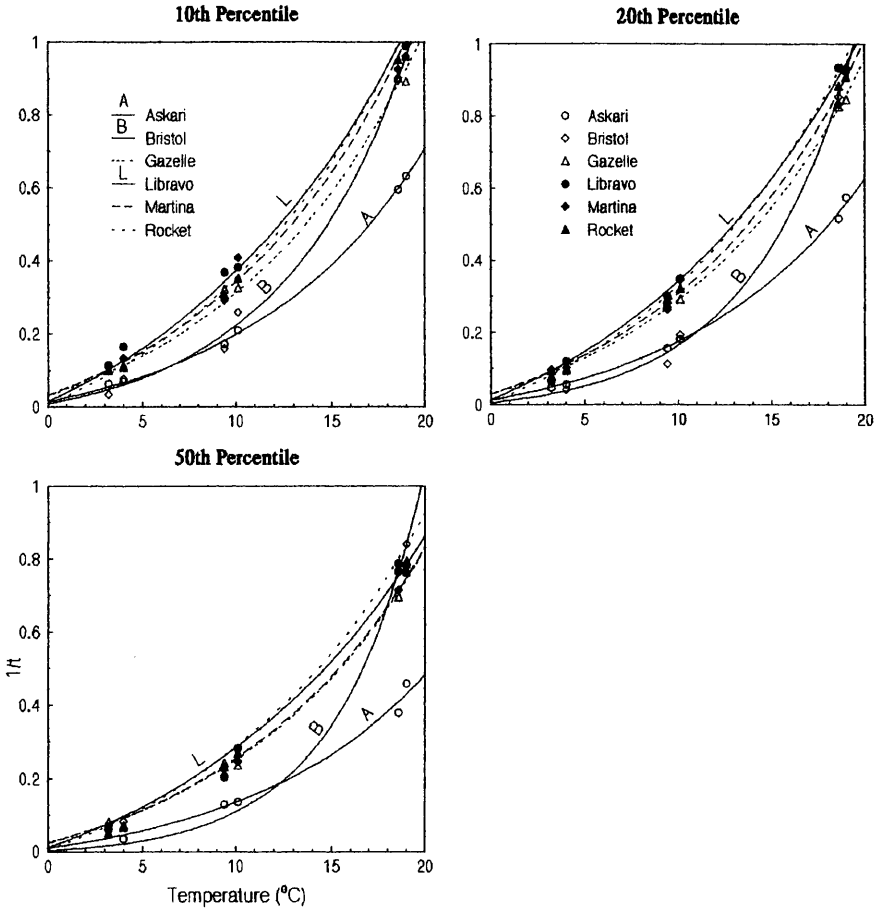



**Figure 3.3 Rates of germination in relation to temperature for the 10th, 20th and 50th percentile groups**

The variation in germination rate between cultivars is now directly compared for the 10th, 20th and 50th percentiles (Figure 3.3). The juxtaposition of curves emphasize that the differences among cultivars change with both percentile and temperature.

### 3.3 Release of dormancy by changing temperature

A procedure was followed to compare the "depth" of secondary dormancy among the cultivars. Temperature, was first raised in small steps, then finally alternated with an amplitude 20°C, until germination of all viable seed was accomplished. The final figures (Table 3.5) demonstrated high viability in the 4°C and 10°C groups, which in all the cases except Askari at 4°C, were either similar to or higher than the 19°C seedlots.

**Table 3.5 Final percentage germination after changing temperature**

| | Nominal Temperature | | | |
|---|---|---|---|---|
| | 4°C | | 10°C | |
| rep | 1 | 2 | 1 | 2 |
| cv. | | | | |
| Askari | 82 | 82 | 88 | 96 |
| Bristol | 98 | 100 | 100 | 98 |
| Gazelle | 94 | 98 | 100 | 94 |
| Libravo | 94 | 92 | 86 | 92 |
| Martina | 100 | 96 | 100 | 100 |
| Rocket | 100 | 96 | 100 | 100 |

Therefore, the seed that did not germinate responded, to a greater or lesser extent among cultivars to an increase in temperature (Figure 3.4). At 4°C and 10C, alternating temperatures were required for Bristol, Libravo, and Martina to complete

germination, with Bristol having large seed fractions of approximately 70% and 60% respectively needing this requirement.

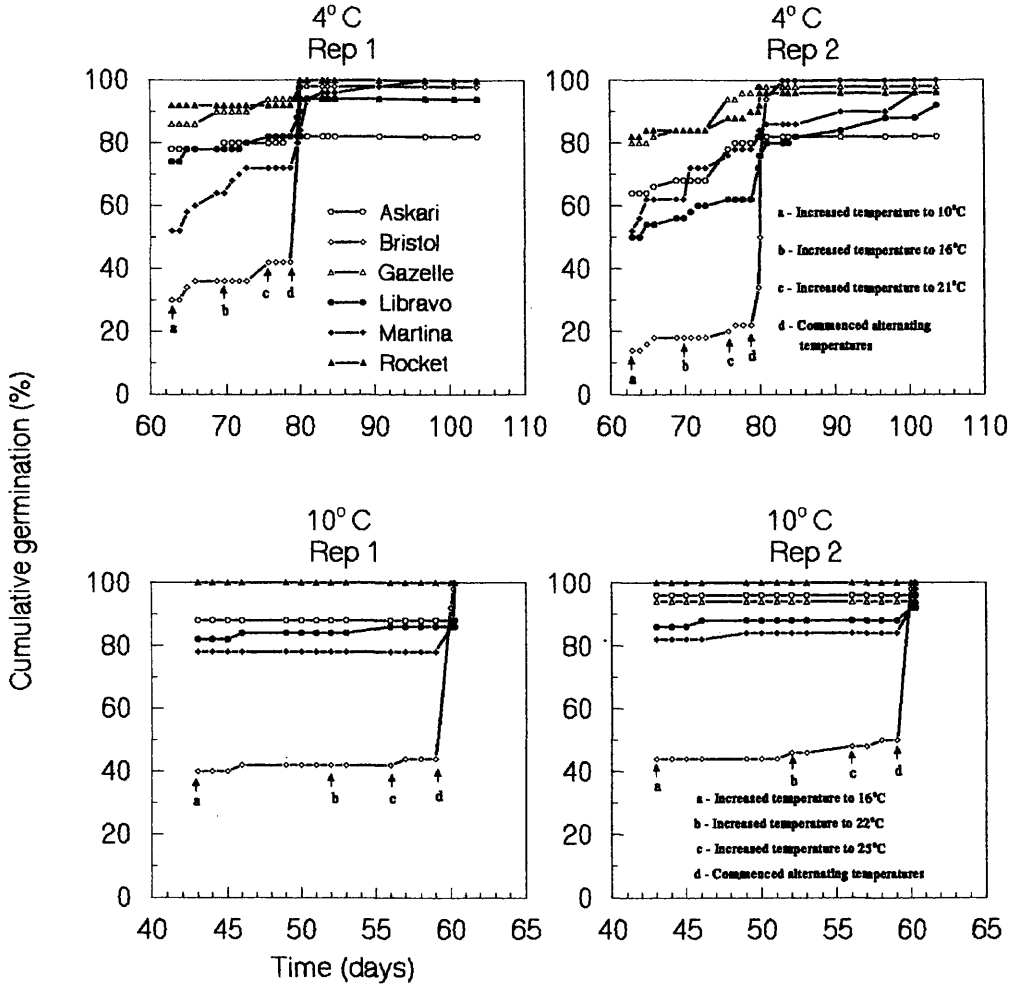



**Figure 3.4 Germination response to changing temperature in the 4°C and 10°C seedlots**

## 3.4 Discussion

Phenotypic variation in temperature-related germination traits was quantified over a much wider range of oilseed rape cultivar that had previously been examined. Time-curves of germination were reduced to a set of quantitative traits that enabled inter-cultivar comparison and are potentially valuable for associating phenotype with genotype. Germination at 19°C indicated that, at least for the cultivars studied, oilseed

rape has little or no primary dormancy. Therefore the main effects captured by the quantitative parameters were a slowing of germination at low temperatures and increasing non-germination at 4°C and 10°C that was due to the induction of secondary dormancy.

Non-linearity in the relation between $1/t$ and temperature caused the most effect at the lower temperatures, where in addition to increasing non-germination there was an enhanced germination rate in the early percentiles, as indicated by the majority of germination-time curves being of or near to the gompertz type. As the temperature increased, the number of generalised logistic curves with a greater $t$ occurred, signifying a more uniform germination rate through the percentiles.

The methodology used generally gave stable and reproducible responses, except in two respects. An inconsistency in the thermal plate was discovered which had important consequences at lower temperatures. A difference of 0.8°C, caused by a horizontal gradient at right angles to the main gradient on the plate, had a large effect on rate and percentage non-germination when the mean temperature was 3-4°C, not far from the base temperature.

The rate-temperature relations of germination described by the exponential models indicated a variation in parameter $a$, common $T_b$ and parameter $b$ for all percentiles in five out of six cultivars. However, the models were limited in their sensitivity by having only three temperature levels to formulate the curves. Where non-germination occurred, mainly at the lower temperatures, missing data points decreased the sensitivity further. In the case of Bristol that was best described by model 2, where parameter $b$ as well as parameter $a$ varied, the insufficient data for 4°C and 10°C exaggerated the curve to such a degree that reliance cannot be made on the values of the model for the later percentiles.

The quantifiable germination traits most pertinent to inter-cultivar discrimination were:

- The variation in parameter *a* between percentiles indicating the degree of heterogeneity.
- The size of the non-germinating fraction at low temperature.
- The temperature stimuli required in breaking secondary dormancy.

These traits were associated in that cultivars variable in parameter *a*, with the exception of Bristol, exhibited a greater degree of dormancy than more uniform cultivars and a greater percentage of seed required an alternating temperature regime to initiate germination. One interesting factor is that, irrespective of cultivar, the greater the size of the non-germinating fraction, the greater the thermal change required for dormancy break. Could seed fraction size of non-germination indicate depth of dormancy within cultivars?

Using these criteria, it was possible to rank the cultivars according to their heterogeneity, Libravo being the most heterogeneous followed by Martina, Rocket, Askari and Gazelle displaying a decreasing range of parameter *a* and non-germination at low temperature. Bristol exhibited a systematic deviation from the rest in that there was a relatively greater suppression of germination at lower constant temperatures than at 19°C: only at lower temperatures great differences in rate and non-germination appeared.

The great variation between cultivars in germination response and dormancy break could have tangible effects in the field. For example, winter oilseed rape, which is the most common type in the United Kingdom, could be affected where autumnal temperatures are around 10°C or below and decreasing.

These conditions could cause irregular and suppressed germination, broadening

emergence time and the flowering period, and thereby increasing the possibility of hybridization with existing feral populations or future crops. A further important implication of the results is that such variability in cultivars would go unnoticed at standard seed testing temperature of 20°C. There is clearly justification for testing for uniformity at lower temperatures as part of routine screening and certification.

## Chapter 4

# GENETIC BASIS FOR THE PHENOTYPIC VARIATION IN GERMINATION WITHIN CULTIVARS

## 4.1 Introduction

In chapter 3, phenotypic variation was in evidence among the cultivars. This chapter is to ascertain whether there is any genetic basis for this variation. Previous studies have found 10°C to be the optimum temperature for genotypic/phenotypic separation in germination for some oilseed rape cultivars (Acharya *et al.*, 1983; Nykiforuk & Johnson-Flanagan, 1994), therefore initial genetic investigation was made at this temperature, only proceeding to the investigation of the phenotypes at the other temperatures if there was sufficient evidence of phenotype/genotype interactions. Leaf samples were taken from specific phenotypes of each cultivar from the laboratory germination test. The DNA was extracted, amplified with two different primers, and the products separated, fixed and stained on polyacrylamide gels.

The total bands scored were between 35 and 40. By scoring a similar approximate total number for each cultivar the degree of polymorphism between cultivars could be obtained. Principal co-ordinate vectors were produced using a Nei & Li similarity matrix and plotted on a 3 dimensional map. The purpose of the 3 dimensional plots, representing genetic distance, was to determine the genetic variation within cultivars and whether there was a genetic base for phenotypic variation in germination.

### 4.2.1 4°C Martina

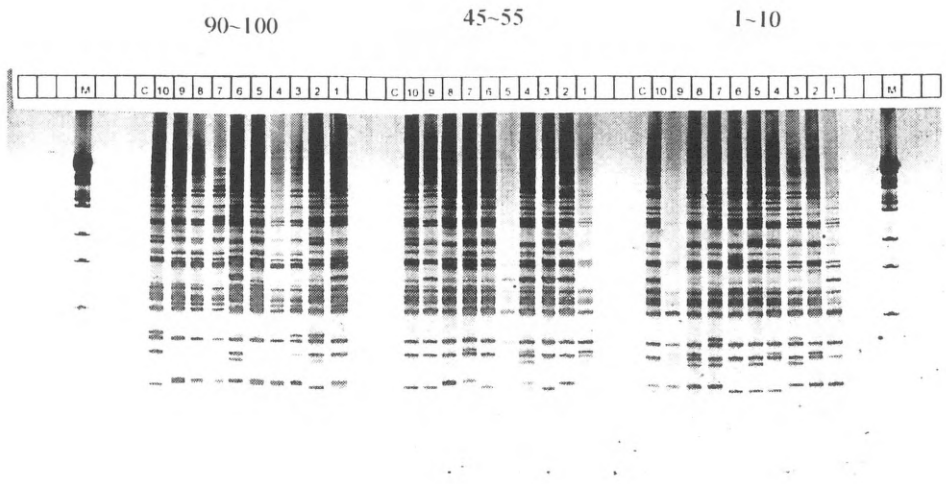
The bands scored from the polyacrylamide gel (Plate 4.1) were constructed into a data matrix (Table 4.1). From the table it was found that 31 out of 35 bands were polymorphic, representing 87% polymorphism. Therefore the main polymorphic bands of interest were associated with distinct differences in phenotype.

4/10/96

PRIMER 1420

GENETIC FINGERPRINTING

4° MARTINA



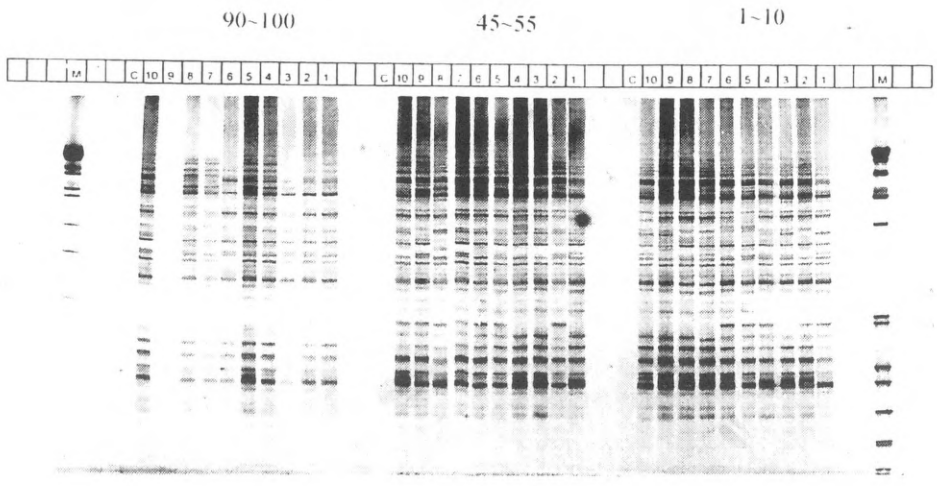

O.S.R. PROJECT 1997

8/11/96

PRIMER 1425

GENETIC FINGERPRINTING

4° MARTINA




O.S.R. PROJECT 1997

**Plate 4.1** - PCR products of 4°C Marina using primers 1420 and 1425

## Table 4.1 4°C Martina data matrix

| Martina 4° | | Late | | | | | | | | | | Middle | | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 18 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 13 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| | 12 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| | 11 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| | 10 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| | 9 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| | 8 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 5 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | 4 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 1425 | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 12 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| | 11 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| | 10 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |

## Table 4.2 Principal bands showing phenotypic differences at 4°C in Martina

| Primer | Band | Late | Middle | Early |
|---|---|---|---|---|
| 1420 | 7 | 6 | 0 | 0 |
| 1420 | 10 | 3 | 6 | 7 |
| 1420 | 14 | 8 | 4 | 1 |
| 1420 | 15 | 7 | 10 | 10 |
| 1425 | 1 | 7 | 1 | 5 |
| 1425 | 2 | 3 | 9 | 9 |
| 1425 | 3 | 7 | 2 | 2 |

Primer 1420 generated four bands that differed between the late, middle and early phenotypes, namely bands 7, 10, 14 and 15 (Table 4.2). Primer 1425 generated three bands that differed between phenotypes, namely band 1, 2 and 3.

Bands 1, 3, 7 and 14 were present in the majority of the late phenotypes in comparison to the other groups, with band 7 only occurring in the lates. In contrast, bands 2, 10 and 15 were generally absent within the late groups.

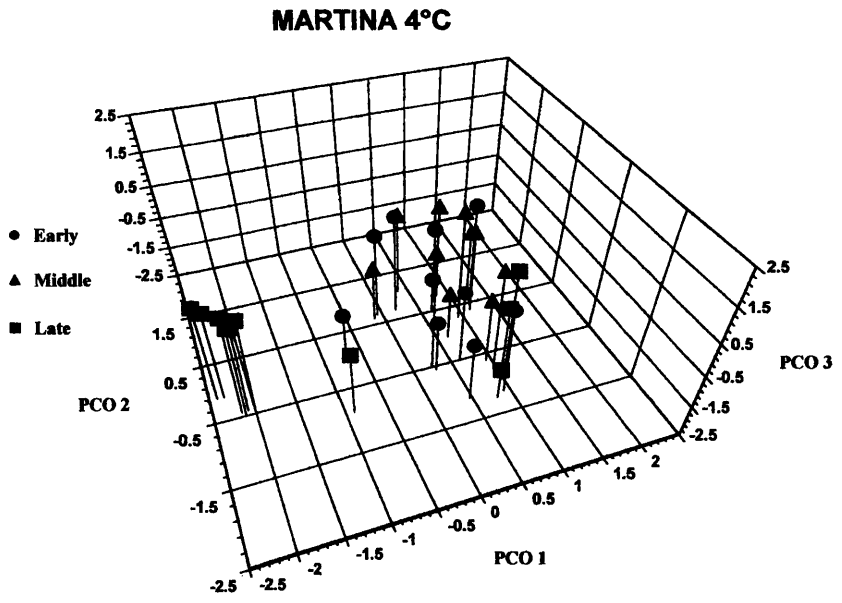



**Figure 4.1 3-Dimensional PCO plot of 4°C Martina**

The three-dimensional plot generated by the Nei & Li similarity matrix (Figure 4.1) shows the population divided into two distinct groups with two individuals midway between. The first group, mostly situated between 0 and 1.0 on the PCO1 axis, is a broad cluster made up of individuals from the early and middle phenotypes plus three from the late phenotypes. There is no indication of differentiation between the early and middle phenotypes in this group, but the three late phenotypes are situated close together. The second grouping, centred between -2.0 and -2.5 on the PCO1 axis, is a tight cluster consisting of six individuals from the late phenotypes only. The two individuals situated midway between the two groups are one early and one late phenotype.

### 4.2.2 10°C Martina

The bands scored from the polyacrylamide gel (Plate 4.2) were constructed into a data matrix (Table 4.3). The number of polymorphic bands was 33 out of a total of 35 representing 94% polymorphism.

**Table 4.3 10°C Martina data matrix**

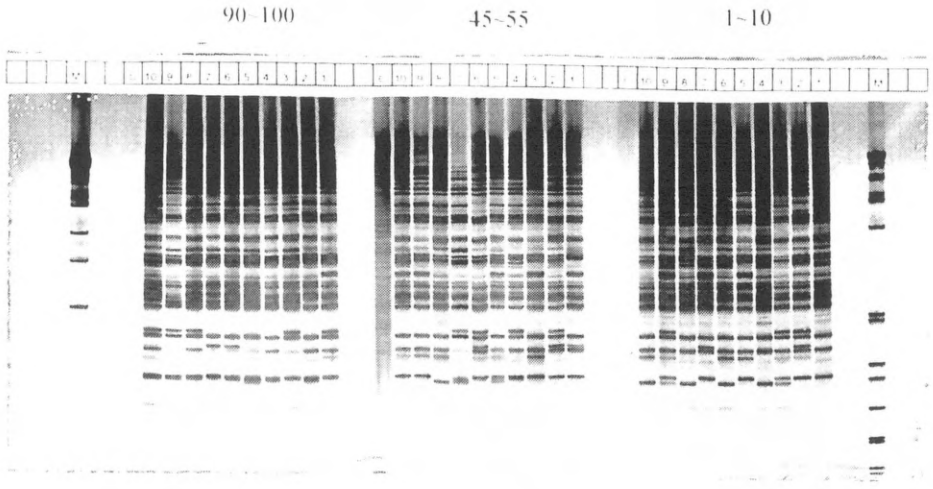
| Martina 10° | | Late | | | | | | | | | | Middle | | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| | 16 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 13 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| | 8 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| | 5 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |
| | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 1425 | 17 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | 12 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| | 10 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |
| | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

19/11/96

PRIMER 1420

GENETIC FINGERPRINTING

10°MARTINA

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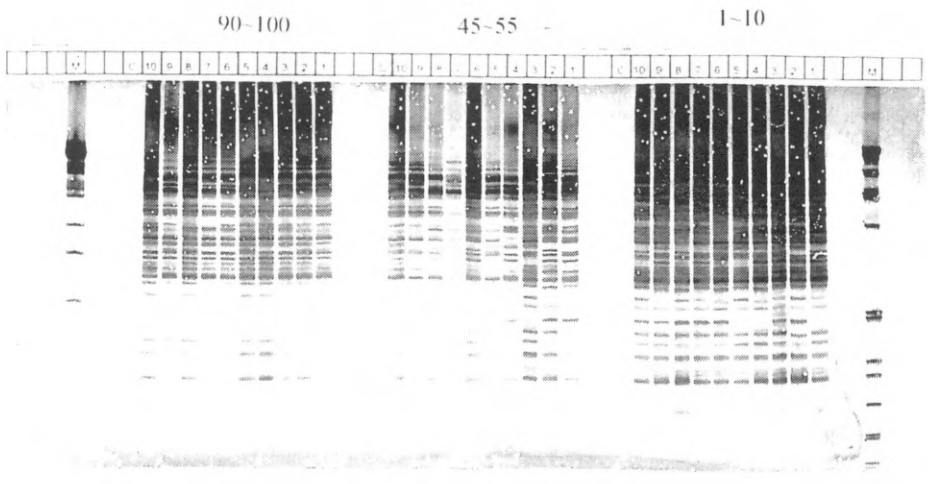
27/11/96

PRIMER 1425

GENETIC FINGERPRINTING

10°MARTINA




O.S.R. PROJECT 1997

**Plate 4.2** - PCR products of 10°C Marina using primers 1420 and 1425

**Table 4.4 Principal bands showing phenotypic differences at 10°C in Martina**

| Primer | Band | Late | Middle | Early |
|---|---|---|---|---|
| 1420 | 7 | 7 | 1 | 0 |
| 1420 | 10 | 1 | 5 | 8 |
| 1420 | 14 | 10 | 4 | 1 |
| 1420 | 15 | 3 | 10 | 10 |
| 1425 | 1 | 9 | 1 | 1 |
| 1425 | 2 | 1 | 9 | 9 |
| 1425 | 3 | 10 | 1 | 1 |

The presence of bands 1, 3, 7 and 14 in the late phenotypes (Table 4.4), increased for all the bands at 10°C in comparison to 4°C. All the late individuals had bands 3 and 14 present, nine individuals had band 1 present, and seven had band 7 present. There was a reduction from five to one early individual having band 1 present. There was also a significant reduction in the number of late individuals having an absence for bands 2, 10 and 15.

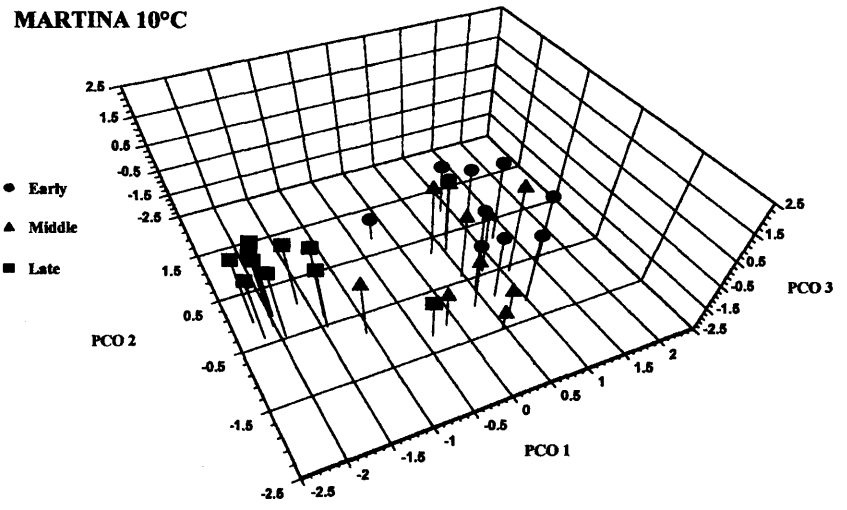



**Figure 4.2 3-Dimensional PCO plot of 10°C Martina**

The 3-D PCO plot of 10°C Martina (Figure 4.2) shows a similarity to 4°C with two genetic populations having a small number of individuals midway. In the first group, the population is made of early and middle phenotypes with no definite

distinction between them. Midway there are one early, two middle and one late phenotype. The second group consists of a cluster of nine late phenotypes.

### 4.2.3 19°C Martina

The bands scored from the polyacrylamide gel (Plate 4.3) were constructed into a data matrix (Table 4.5). The number of polymorphic bands was 29 out of a total of 35 representing 83% polymorphism.

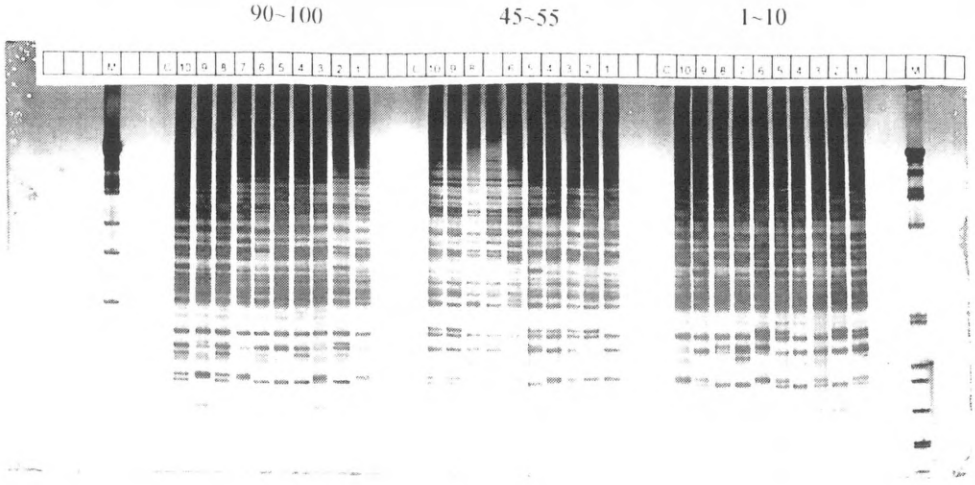
**Table 4.5 19°C Martina data matrix**

| Martina 19° | | Late | | | | | | | | | | Middle | | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 18 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 16 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| | 14 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| | 11 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| | 5 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 |
| | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| 1425 | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| | 13 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| | 12 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| | 7 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

3/12/96
PRIMER 1420

GENETIC FINGERPRINTING

19° MARTINA

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5/12/96
PRIMER 1425

GENETIC FINGERPRINTING

19° MARTINA

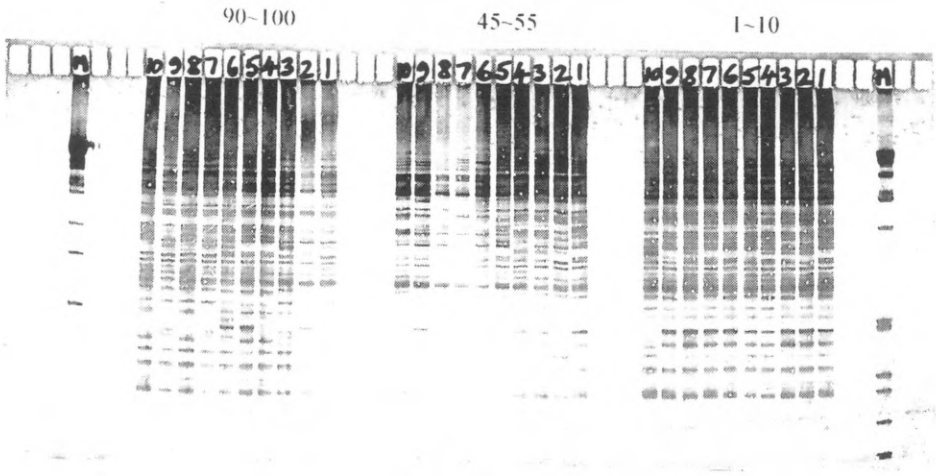


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**Plate 4.3** - PCR products of 19°C Marina using primers 1420 and 1425

**Table 4.6 Principal bands showing phenotypic differences at 19°C in Martina**

| Primer | Band | Late | Middle | Early |
|---|---|---|---|---|
| 1420 | 7 | 4 | 1 | 1 |
| 1420 | 10 | 3 | 2 | 4 |
| 1420 | 14 | 4 | 3 | 4 |
| 1420 | 15 | 8 | 10 | 9 |
| 1425 | 1 | 5 | 4 | 1 |
| 1425 | 2 | 5 | 6 | 9 |
| 1425 | 3 | 5 | 4 | 1 |

The occurrence of bands 1, 3, 7 and 14 in late phenotypes, was substantially reduced in comparison with the other two temperatures (Table 4.6). For all these bands the reduction was approximately 50% from 10°C to 19°C. Similarly there was an increase in the number of individuals having the bands 2, 10 and 15 present in comparison to the other two temperatures. There were no distinct differences between the late, middle and early groups for bands 10, 14 and 15 at 19°C.

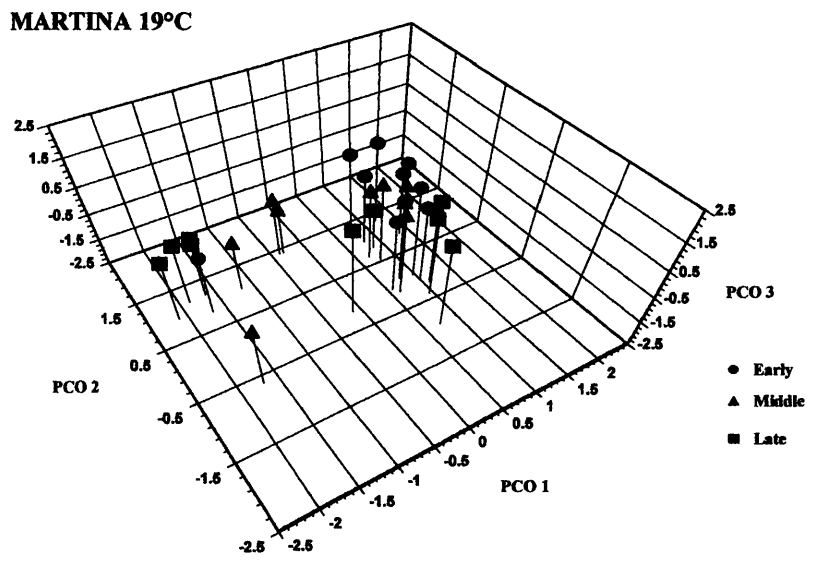



**Figure 4.3 3-Dimensional PCO plot of 19°C Martina**

The 3-D PCO plot for 19°C (Figure 4.3) indicates a substantial shift in the genetic population. The first group, which still remains a broad cluster, contains five late phenotypes among the early and middle phenotypes. There are four individuals situated midway between the two groups, consisting of one late and three middle phenotypes. The second group consists of four late, one middle and one early phenotype. The geographic positioning of the second group indicates a shift in the PCO 2 vector to +1.0, where as the grouping at 4°C and 10°C was centred around 0.0 on the PCO 2 axis. The positioning of the phenotypic groups at 19°C shows more late individuals going towards the first group and more middle individuals going towards the second group, whilst the early individuals remaining where they are, resulting in the distinctiveness between phenotypic groups being substantially reduced in comparison with 4°C and 10°C.

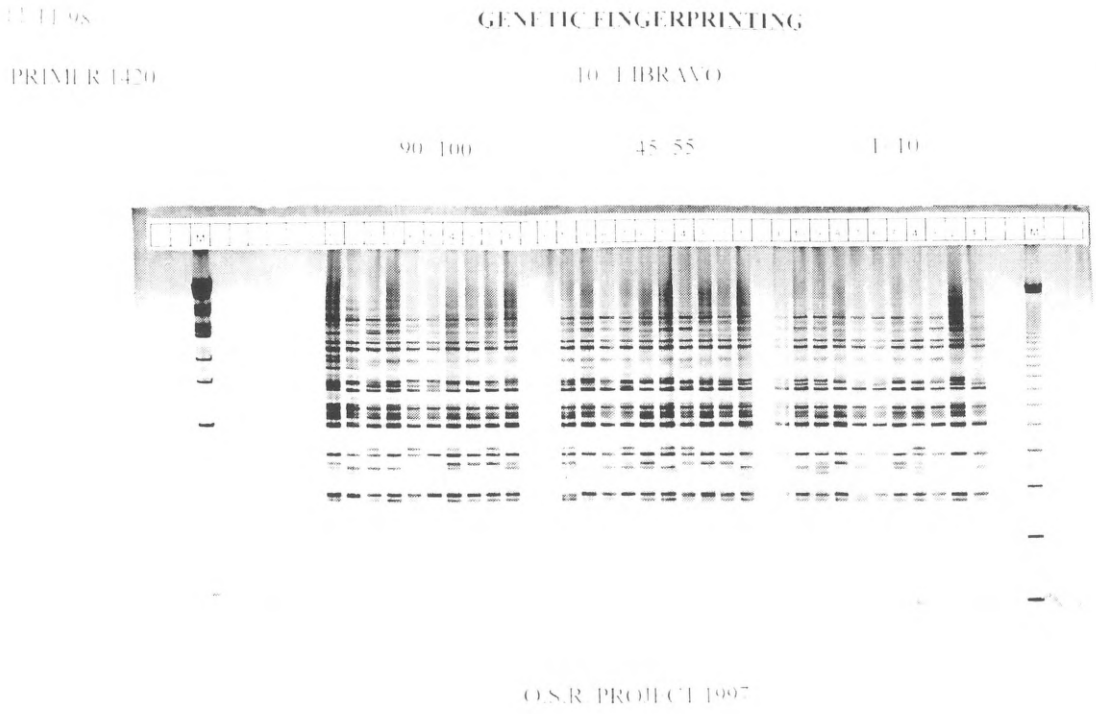
## 4.3 Other varieties

The best discrimination is at 10°C, so all other varieties are done at 10°C only.

### 4.3.1 10°C Libravo

The bands scored from the polyacrylamide gel (Plate 4.4) were constructed into a data matrix (Table 4.7). The number of polymorphic bands was 12 out of a total of 40, representing 30% polymorphism.

There were three bands that might possibly link with phenotype. From primer 1420, band 7 was present in seven late, five middle and one early phenotypes, and band 21 was present in five late, two middle and two early phenotypes. From primer 1425, band 11 was present in five late, eight middle and ten early phenotypes. All the other polymorphic bands did not show any differentiation between phenotypic groups.



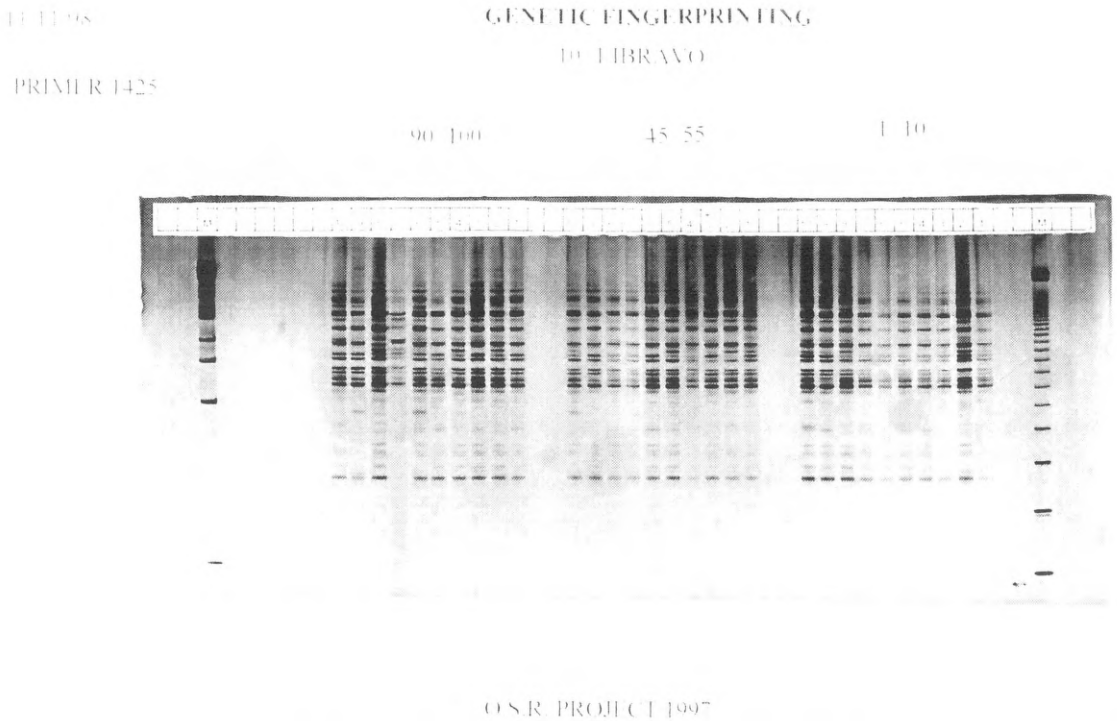
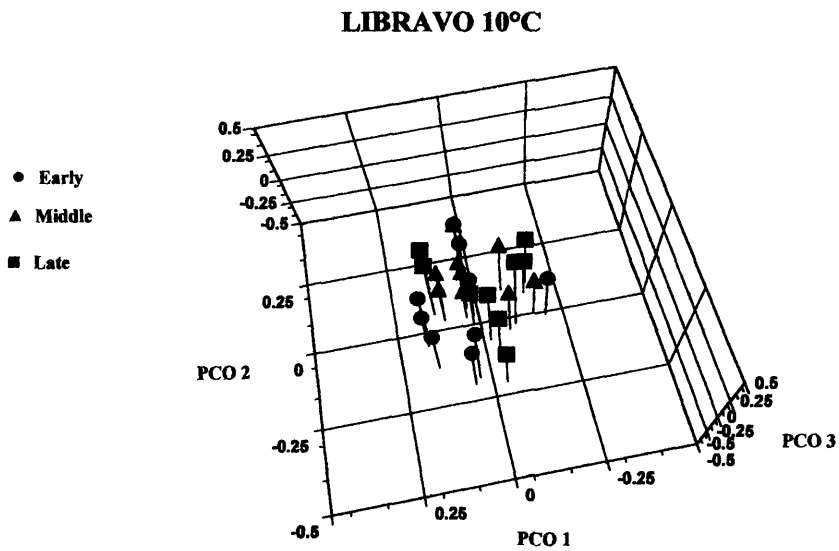




**Plate 4.4** - PCR products of 10°C Libravo using primers 1420 and 1425

## Table 4.7 10°C Libravo data matrix

| Libravo 10° | | Late | | | | | | | | | | Middle | | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 22 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 21 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| | 20 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| | 14 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1425 | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |




## Figure 4.4 3-Dimensional PCO plot of 10°C Libravo

The 3-D PCO plot of 10°C Libravo (Figure 4.4) shows a smaller genetic spread within the population in comparison with Martina. The circular cluster does not show any phenotypic separation, though there is a tendency for the late phenotypes to be situated in the eastern half (PCO 1 >0.0) and the early phenotypes to be situated in the western half (PCO 1 <0.0) of the cluster.

### 4.3.2 10°C Bristol

The bands scored from the polyacrylamide gel (Plate 4.5) were constructed into a data matrix (Table 4.8). The number of polymorphic bands was 9 out of a total of 40 representing 22% polymorphism. In this case, all the polymorphism occurred in one individual only.

**Table 4.8 10°C Bristol data matrix**

| Bristol 10° | | Late | | | | | | | | | | Middle | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 20 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1425 | 20 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

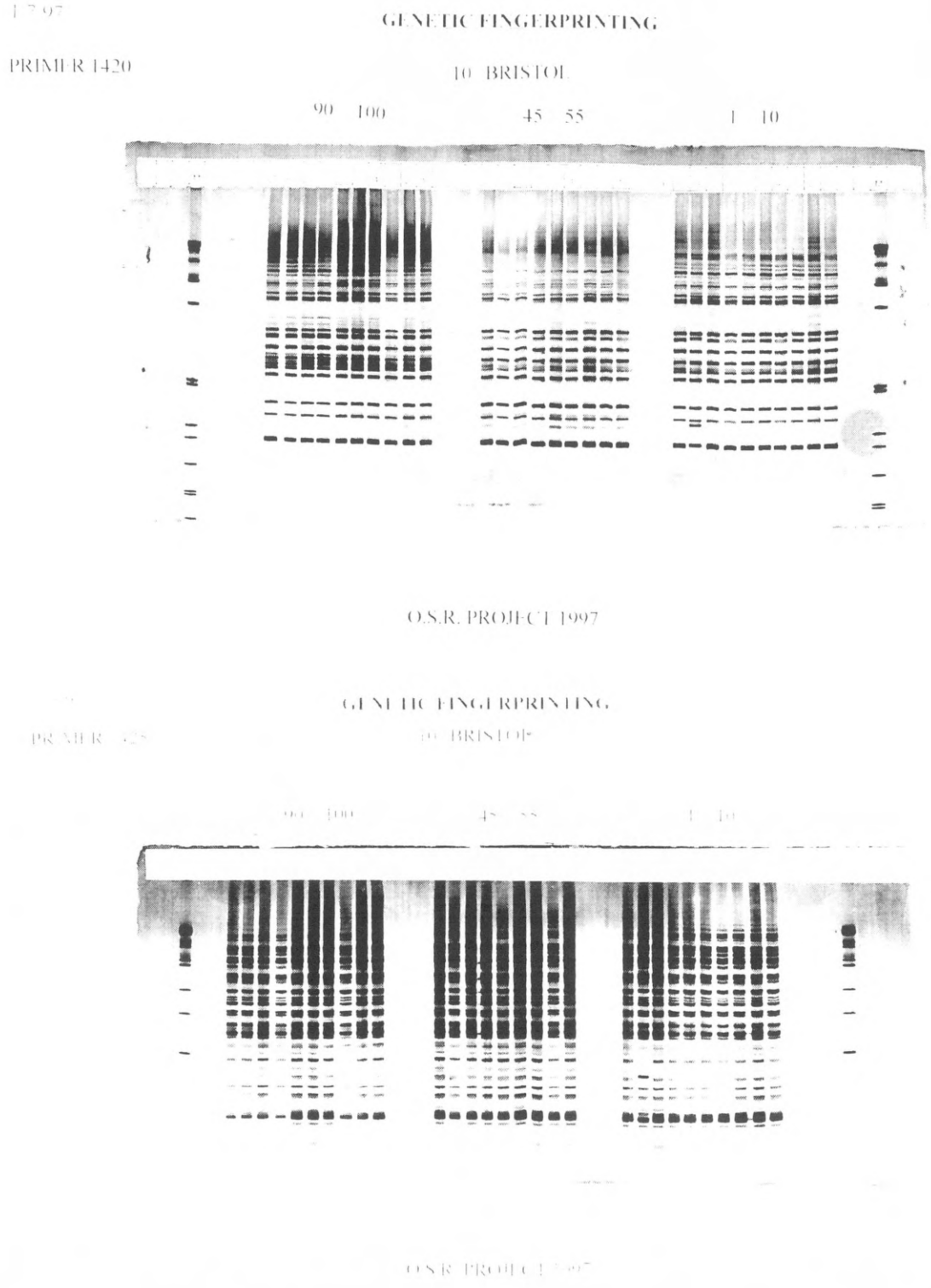
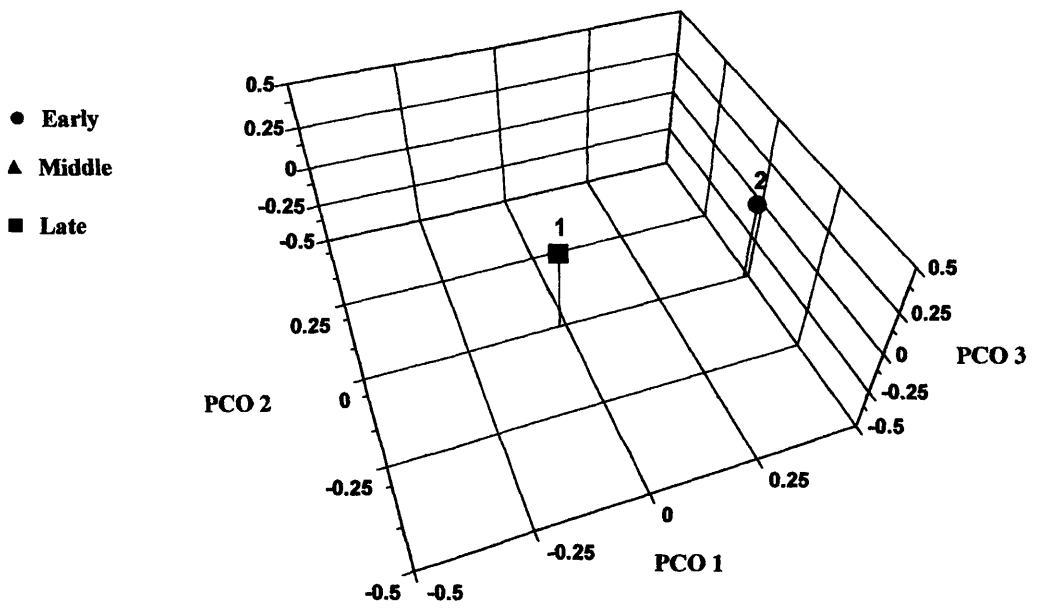



**Plate 4.5** - PCR products of 10°C Bristol using primers 1420 and 1425




1: All plants except E9 2: E9

**Figure 4.5 3-Dimensional PCO plot of 10°C Bristol**

The 3-D PCO plot of 10°C Bristol (Figure 4.5) shows that all the plants with the exception of an early phenotype are invariant for the primers used.

### 4.3.3 10°C Gazelle

The bands scored from the polyacrylamide gel (Plate 4.6) were constructed into a data matrix (Table 4.9). The number of polymorphic bands was 1 out of a total of 37 representing 3% polymorphism. The numbers of individual plants were reduced due to aphid attacks on the young plants during propagation, therefore only five late and nine middle phenotypes were available. There was only one polymorphic band, band 11 of primer 1420, which was present in four late, three middle and two early phenotypes.

17-11-98

GENETIC FINGERPRINTING

PRIMER 1420

10° GAZELLE

90-100 45-55 1-10

O.S.R. PROJECT 1997

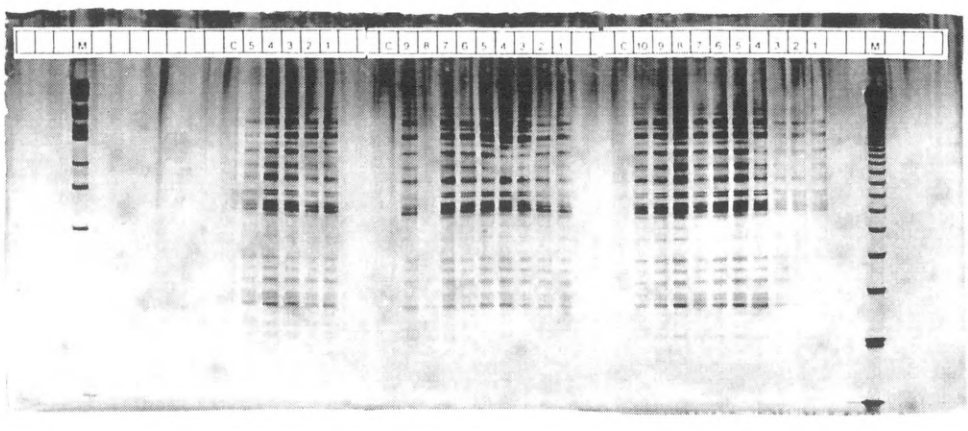
4-9-98

GENETIC FINGERPRINTING

PRIMER 1425

10° GAZELLE

90-100 45-55 1-10



O.S.R. PROJECT 1997

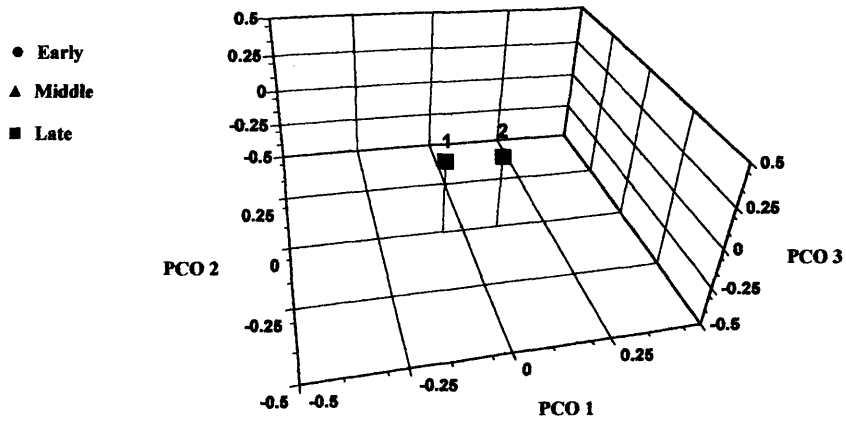
**Plate 4.6** - PCR products of 10°C Gazelle using primers 1420 and 1425

**Table 4.9 10°C Gazelle data matrix**

| Gazelle 10° | | Late | | | | | Middle | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 5 | 4 | 3 | 2 | 1 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1425 | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

The 3-D PCO plot of 10°C Gazelle (Figure 4.6) shows the population divided into two groups depending on whether they displayed band 11 of primer 1420. The individuals with this band present are in population group 1, those with it absent are in population 2. There is no indication of phenotypic differentiation between the two groups, even though there is a tendency towards later germinators in the first group and early germinators in the second group.

GAZELLE 10°C




1: E1, E3
M2, M6, M7
L1, L2, L3, L5

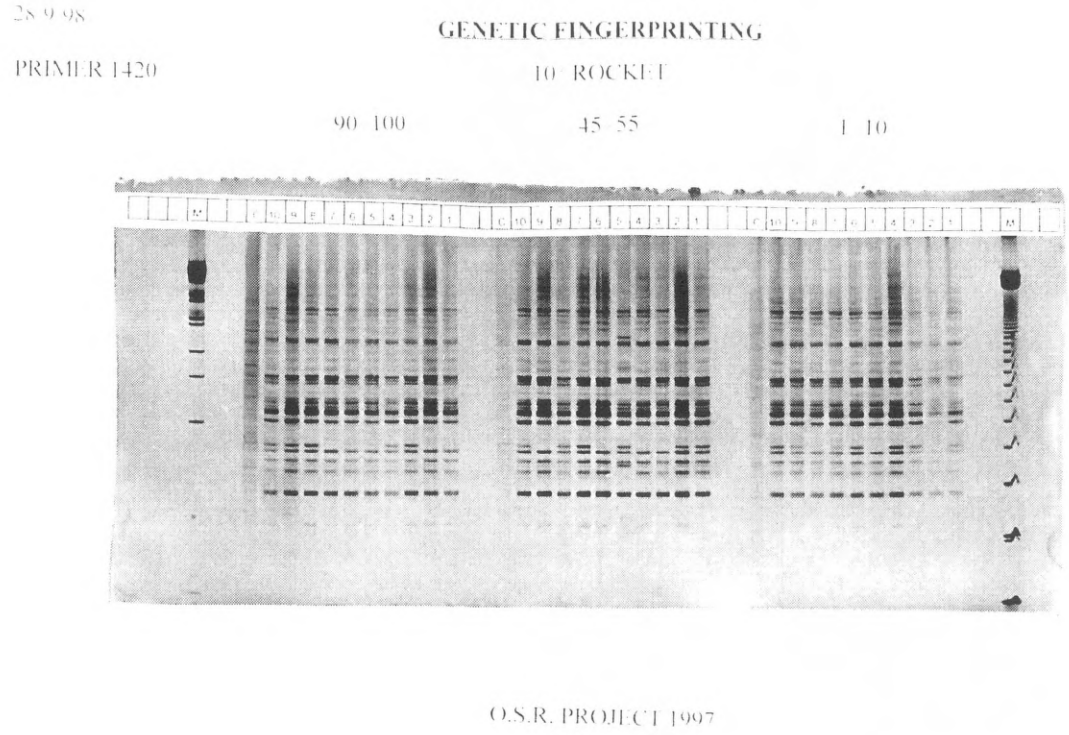
2: E2, E4, E5, E6, E7, E8, E9, E10
M1, M3, M4, M5, M8, M9
L4

**Figure 4.6 3-Dimensional PCO plot of 10°C Gazelle**

### 4.3.4 10°C Rocket

The bands scored from the polyacrylamide gel (Plate 4.7) were constructed into a data matrix (Table 4.10). The number of polymorphic bands was 6 out of a total of 36 representing 17% polymorphism.

In three out of the six polymorphic bands, only one individual showed variation. The other three polymorphic bands were, band 6 of primer 1420 and bands 7 and 8 of primer 1425. Band 6 was present in five late, three middle and two early phenotypes. Band 7 was present in five late, ten middle and six early, while band 8 was present in seven early, four middle and five early phenotypes. There was no obvious phenotypic weighting for any of the significant bands.



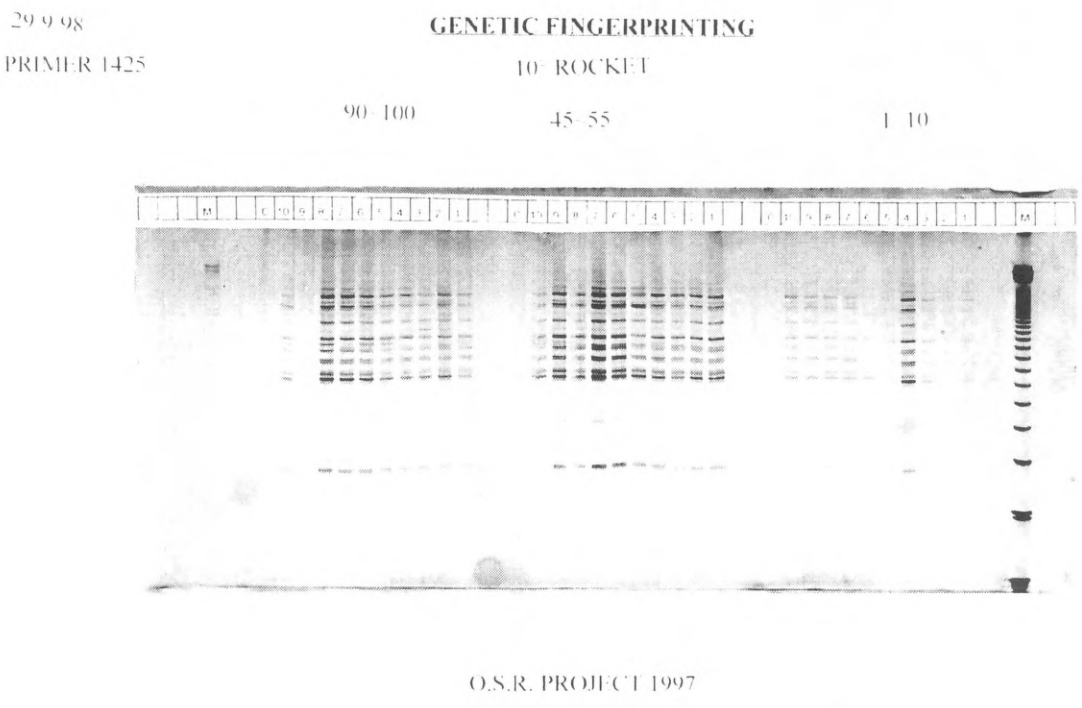



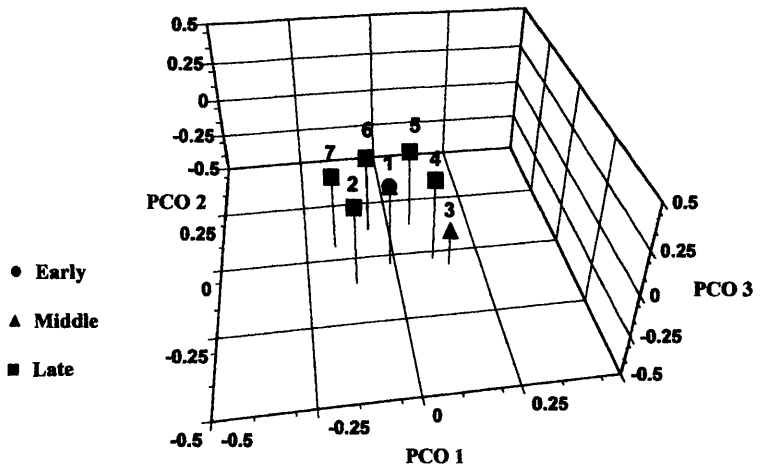

**Plate 4.7** - PCR products of 10°C Rocket using primers 1420 and 1425

**Table 4.10 10°C Rocket data matrix**

| Rocket 10° | | Late | | | | | | | | | | Middle | | | | | | | | | | Early | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 20 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1425 | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| | 7 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

The 3-D PCO plot of 10°C Rocket (Figure 4.7) showed a small cluster. Population 3 consisted of an isolated individual of a middle phenotype that had three bands different from the rest of the population. The other six groups were formed from the other three polymorphic bands. There is no leaning in any one group towards earliness or lateness of germination; late, middle and early phenotypes being represented in groups four and five respectively.

**ROCKET 10°**




| | | | |
|---|---|---|---|
| 1: E4 | 2: E1, E6, E7 | 3: | 4: E3, E8, E9, E10 |
| M8 | | M5 | M1, M2, M6, M7 |
| | L8, L9, L10 | | L3, L4 |
| 5: E2 | 6: E5 | 7: | |
| M3 | | M4, M9 | |
| L2 | L1, L5 | L6, L7 | |

**Figure 4.7 3-Dimensional PCO plot of 10° Rocket**

### 4.3.5 10°C Askari

The bands scored from the polyacrylamide gel (Plate 4.8) were constructed into a data matrix (Table 4.11). The number of polymorphic bands was 4 out of a total of 35 representing 11% polymorphism. The late group was restricted to five individuals because of aphid attack mentioned for previous cultivars. The four polymorphic bands were band 3, 4, 5 and 6 of primer 1420. Band 3 and 4 consisted of one individual each, in the early group having a band present or absent. Band 5 was present in three late, four middle and six early phenotypes while band 6 was present in two late, six middle and four early phenotypes.

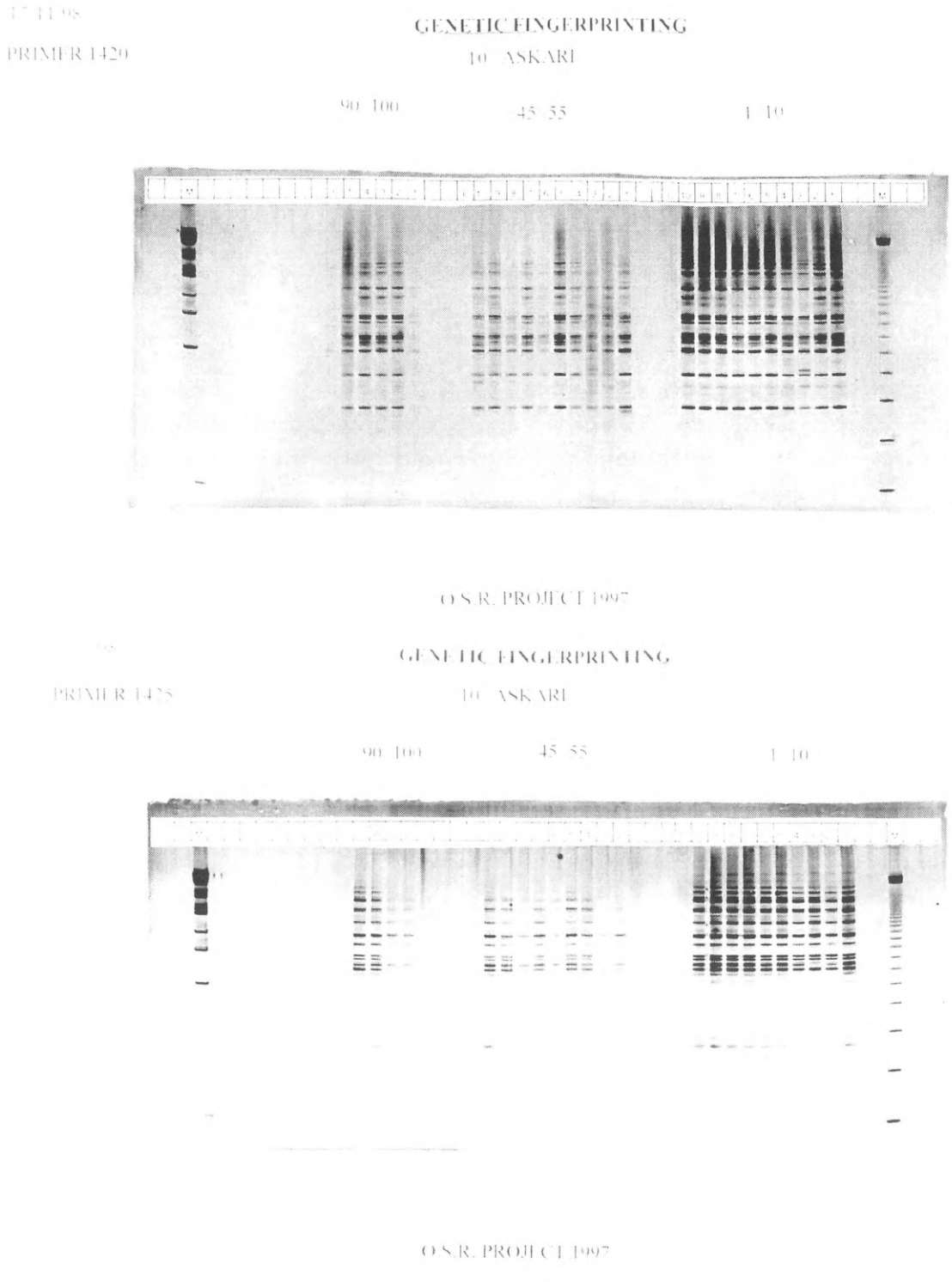


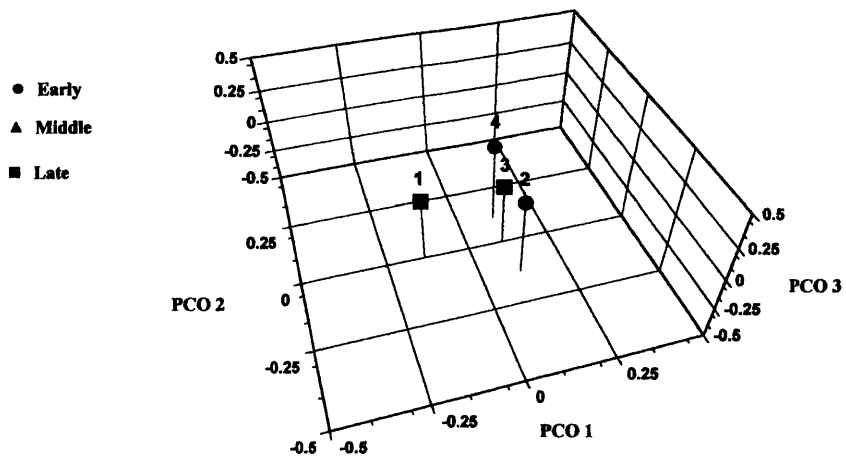

**Plate 4.8** - PCR products of 10°C Askari using primers 1420 and 1425

**Table 4.11 10°C Askari data matrix**

| Askari 10° | | Late | | | | | Middle | | | | | | | | | Early | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed | | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Primer | Band | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1420 | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| | 5 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1425 | 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

The 3-D PCO plot for 10° Askari (Figure 4.8) had two distinct populations, 1 and 3, as determined by bands 5 and 6. Within these two populations there was a similar representation of all phenotypes.

The populations 2 and 4 were single individuals from the early phenotypes, which were separated by bands 3 and 4.




1: E1, E5, E6, E7, M2, M3, M5, M7, M10 L4, L5

2: E10

3: E2, E4, E8, E9 M1, M4, M6, M8, M9 L1, L2, L3

4: E3

**Figure 4.8 3-Dimensional PCO plot of 10°C Askari**

## 4.4 Discussion

The cultivars selected were based upon the degree of heterogeneity found from previous work (Charters *et al.*, 1996a). In comparison with this previous work the number of polymorphic bands found using primer 1420 increased for all the cultivars except Libravo. The greatest increase of polymorphism occurred in Martina where eighteen bands in contrast to nine were found. In the other cultivars up to four additional polymorphic bands were found, with the loss of two bands from Libravo. The additional bands were revealed as a consequence of the refinements in technique in the present study.

Martina exhibited the greatest polymorphism from the six cultivars studied having an average of 89% at all three temperatures. From a total of 90 Martina plants screened at the three temperatures, only two individuals were identical using primers 1420 and 1425, indicating a highly heterogeneous population. Genetic association with germination phenotypes was evident, only in Martina when the 3-D PCO plots confirmed this linkage with the separation of individuals into two populations. The

first population mainly consisted of early and middle phenotypes in a broad cluster while the second population was composed of mainly late phenotypes in a tight group. The temperature of 19°C produced the least distinction between phenotypes possibly because germination at this temperature was very fast. Therefore a blurring between different genetic populations might have occurred. At 4°C, while the rate of germination was much slower with 50% of the population being non-germinators, discrimination between phenotype was clearer.

The temperature of 10°C, reported to be the optimum temperature for genotype/phenotype separation (Acharya *et al.*, 1983; Nykiforuk & Johnson-Flanagan, 1994), seems to bear this hypothesis out. Only 20% of the population was non-germinators, the first population group were made up entirely of early and middle germinators whilst the second population group were made up of nine late germinators with one late phenotype midway, giving a stronger clearer distinction of genotype being synchronized with phenotype.

The other five cultivars had substantially less polymorphism within their genetic base. Libravo had the next highest polymorphism of 30% but no indication that phenotypic germination was linked to genotypes of individuals. The reduction of polymorphism within the remaining cultivars also saw the increase of identical individuals; hence no genotypic/phenotypic interactions were observed. Rocket and Askari had seven and four genotypes respectively, Gazelle's population was made up of two genotypes in equal proportions and individuals of Bristol were all identical with the exception of one. However, the degree of polymorphism cannot be described simply by the number of polymorphic bands found within a population, as in the case of Bristol only one individual was responsible for seven polymorphic bands.

The cultivars can now be ranked according to the genetic heterogeneity found, Martina being the most heterogeneous followed by Libravo, then Rocket, Askari, Bristol and Gazelle.

## Chapter 5

# OVER-WINTER FIELD EMERGENCE

## 5.1 Introduction

The object of this experiment was to observe the effects of over-wintering on the emergence of the six cultivars of oilseed rape, and then to assess whether the varietal differences were carried over from thermal plate to field. The effect of decreasing late autumnal temperatures should produce different time emergence profiles between cultivars consistent with their germination traits (Squire, 1999). Moreover, periods of cold weather occurring during these trials could induce secondary dormancy, thereby giving the opportunity to witness the post-winter response of dormancy break among cultivars.

The three dates of sowing had to be dependant on prevailing and expected weather bearing in mind that previous winters had been mild with short periods of freezing conditions. Present day weather forecasting is limited in giving accurate weather conditions for days ahead rather than weeks ahead. If sowing was made too early prior to the onset of cold conditions, then nearly 100% emergence would occur and there would be no seeds left for over-wintering dormancy to occur. If sowing was made too late during a prolonged period of cold, no emergence would occur. The desired ideal was to sow under favourable growing temperatures, but have the daily average temperature slowly drop as time progressed so that induction of dormancy due to low temperature would occur during the emergence phase.

## 5.2 Soil temperature and emergence

The first two sowings were made on 31 October 1997 and 17 November 1997 respectively when the average soil temperatures were around 7°C. It was decided to delay the third sowing until 21 January 1998 when it was much cooler. The soil moisture content was at full field capacity throughout (Appendix 3).

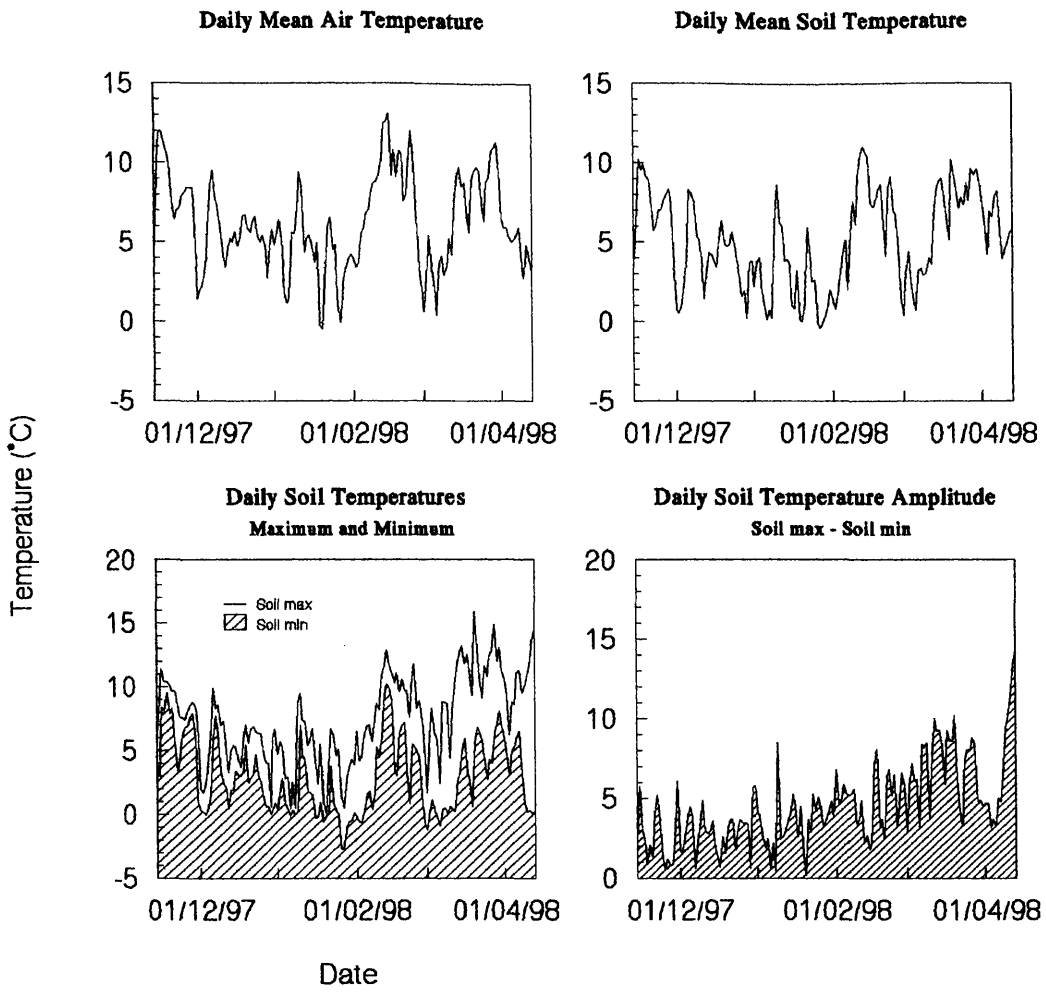



**Figure 5.1 Daily average temperatures during emergence trials**

The period October 1997 to March 1998 saw average soil temperatures of 1.8°C above the 30 year long term average, the months of November and February having the highest rises of 2.6°C and 4.1°C respectively. There were five distinct cold periods of three days or more during the experiment when temperatures dropped to below 2°C (Figure 5.1), their middle dates occurring on 2 December 1997, 6 January 1998, 20 January 1998, 27 January 1998 and 1 March 1998.

The soil temperatures were averaged for each cultivar over three distinct phases in emergence: first ten days (T1); first 10% of emergence (T2); after 70 days (T3) (Table 5.1).

**Table 5.1 Average soil temperatures over first ten days (T1), first 10% of emergence (T2) and after 70 days (T3)**

| 1st Sowing | T1 | T2 | T3 |
|---|---|---|---|
| Askari | 7.5 | 6.2 | 5.1 |
| Bristol | 7.5 | 7.3 | 5.2 |
| Gazelle | 7.5 | 6.9 | 5.2 |
| Libravo | 7.5 | 7.3 | 5.1 |
| Martina | 7.5 | 7.0 | 5.2 |
| Rocket | 7.5 | 7.3 | 5.2 |
| 2nd Sowing | T1 | T2 | T3 |
| Askari | 7.6 | 4.4 | 4.0 |
| Bristol | 7.6 | 5.3 | 4.1 |
| Gazelle | 7.6 | 5.8 | 4.1 |
| Libravo | 7.6 | 5.4 | 4.1 |
| Martina | 7.6 | 5.6 | 4.0 |
| Rocket | 7.6 | 5.2 | 4.0 |
| 3rd Sowing | T1 | T2 | T3 |
| Askari | 1.6 | * | 5.4 |
| Bristol | 1.6 | 4.8 | 5.6 |
| Gazelle | 1.6 | 4.5 | 5.5 |
| Libravo | 1.6 | 4.8 | 5.6 |
| Martina | 1.6 | 4.8 | 5.4 |
| Rocket | 1.6 | 4.9 | 5.6 |

The average soil temperatures for the first and second sowings at the T1 stage were very similar, but while this temperature was maintained for the first sowing up to the first 10% of emergence, a spell of cold weather centred around 2 December 1997 reduced the average temperature of the second sowing between T1 and T2 by approximately 2°C. The overall difference in temperature between the two sowings, which were started 17 days apart, was 1°C.

In contrast, the third sowing, which commenced in January, had a low average temperature for the first ten days due to spells of cold weather. The temperature increased between T1 and T3, the overall average temperature reaching 5.5°C, which was the highest for all three sowings.

Comparison of emergence from all three sowings (Table 5.2) indicates that the first sowing generally achieved the highest emergence, followed by the second and third respectively.

**Table 5.2 Pre-winter emergence in field trials**

| 1st Sowing | Rep 1 | Rep 2 | Rep 3 | Rep 4 |
|---|---|---|---|---|
| Askari | 11 | 10 | 12 | 7 |
| Bristol | 68 | 79 | 84 | 83 |
| Gazelle | 100 | 100 | 92 | 90 |
| Libravo | 45 | 60 | 50 | 46 |
| Martina | 85 | 75 | 86 | 75 |
| Rocket | 68 | 60 | 53 | 71 |
| **2nd Sowing** | **Rep 1** | **Rep 2** | **Rep 3** | **Rep 4** |
| Askari | 14 | 19 | 19 | 18 |
| Bristol | 63 | 63 | 70 | 52 |
| Gazelle | 84 | 83 | 79 | 80 |
| Libravo | 44 | 32 | 51 | 49 |
| Martina | 63 | 62 | 61 | 56 |
| Rocket | 50 | 57 | 37 | 40 |
| **3rd Sowing** | **Rep 1** | **Rep 2** | **Rep 3** | **Rep 4** |
| Askari | 0 | 3 | 3 | 1 |
| Bristol | 59 | 76 | 77 | 54 |
| Gazelle | 42 | 31 | 42 | 39 |
| Libravo | 31 | 33 | 34 | 26 |
| Martina | 31 | 46 | 44 | 43 |
| Rocket | 36 | 29 | 43 | 34 |

Inter-cultivar variation was evident, Gazelle and Askari being the best and poorest performers respectively. Most cultivars had a similar ranking between sowings, the notable exception being Bristol, which had its highest emergence in the third sowing. The differences between replicates for sowing 1, i.e. the difference between the highest and lowest emergence percentage for the four replicates, ranged from 10% for Gazelle to 18% for Rocket. In sowing 2, there was a 5% difference for

Gazelle and 19% difference for Libravo. In Sowing 3, Libravo had 8% and Bristol 22% respectively.

## 5.3 Profiles of emergence with time

Cumulative emergence curves were fitted to the emergence data sets based on generalised logistic, logistic or gompertz functions using Genstat V.

**Table 5.3 Generalised logistic, logistic and gompertz functions for time to emergence curves in all sowings**

| Sowing | cv. | $b$ | $m$ | $y_{max}$ | $t$ | type | % accounted for |
|---|---|---|---|---|---|---|---|
| 1 Rep 1 | Askari | 0.189 | 30.5 | 11.0 | 0 | gompertz | 99.4 |
| 1 Rep 2 | Askari | 0.075 | 35.2 | 10.3 | 0 | gompertz | 97.3 |
| 1 Rep 3 | Askari | 0.185 | 34.1 | 12.1 | 0.748 | glogistic | 99.4 |
| 1 Rep 4 | Askari | 0.083 | 37.1 | 7.3 | 0 | gompertz | 98.2 |
| 1 Rep 1 | Bristol | 0.368 | 21.0 | 67.7 | 0 | gompertz | 99.9 |
| 1 Rep 2 | Bristol | 0.700 | 21.1 | 77.2 | 0 | gompertz | 99.5 |
| 1 Rep 3 | Bristol | 0.466 | 20.2 | 81.2 | 0 | gompertz | 99.6 |
| 1 Rep 4 | Bristol | 0.486 | 20.4 | 81.4 | 0 | gompertz | 99.5 |
| 1 Rep 1 | Gazelle | 2.334 | 17.4 | 99.8 | 0.011 | glogistic | 100.0 |
| 1 Rep 2 | Gazelle | 1.397 | 17.9 | 98.5 | 0.011 | glogistic | 99.9 |
| 1 Rep 3 | Gazelle | 1.464 | 17.8 | 91.4 | 0.011 | glogistic | 99.9 |
| 1 Rep 4 | Gazelle | 1.238 | 17.7 | 90.0 | 0.011 | glogistic | 99.9 |
| 1 Rep 1 | Libravo | 0.474 | 20.1 | 43.8 | 0 | gompertz | 99.3 |
| 1 Rep 2 | Libravo | 0.209 | 22.2 | 57.5 | 0 | gompertz | 99.1 |
| 1 Rep 3 | Libravo | 0.236 | 22.6 | 48.5 | 0 | gompertz | 99.5 |
| 1 Rep 4 | Libravo | 0.325 | 21.6 | 43.8 | 0 | gompertz | 99.5 |
| 1 Rep 1 | Martina | 0.413 | 18.7 | 82.4 | 0 | gompertz | 99.4 |
| 1 Rep 2 | Martina | 0.358 | 18.8 | 72.7 | 0 | gompertz | 99.6 |
| 1 Rep 3 | Martina | 0.394 | 18.7 | 83.8 | 0 | gompertz | 98.9 |
| 1 Rep 4 | Martina | 0.238 | 20.5 | 74.4 | 0 | gompertz | 99.7 |
| 1 Rep 1 | Rocket | 0.259 | 21.2 | 66.6 | 0 | gompertz | 99.6 |
| 1 Rep 2 | Rocket | 0.365 | 21.2 | 58.0 | 0 | gompertz | 99.5 |
| 1 Rep 3 | Rocket | 0.339 | 20.8 | 52.4 | 0 | gompertz | 99.7 |
| 1 Rep 4 | Rocket | 0.317 | 21.6 | 69.6 | 0 | gompertz | 99.7 |
| 2 Rep 1 | Askari | 0.129 | 38.2 | 14.3 | 0 | gompertz | 99.4 |
| 2 Rep 2 | Askari | 2.199 | 56.3 | 18.5 | 19 | glogistic | 99.4 |
| 2 Rep 3 | Askari | 0.078 | 44.3 | 19.9 | 0 | gompertz | 99.2 |
| 2 Rep 4 | Askari | 0.135 | 41.1 | 18.4 | 0 | gompertz | 99.7 |
| 2 Rep 1 | Bristol | 0.122 | 32.1 | 62.8 | 0 | gompertz | 99.9 |
| 2 Rep 2 | Bristol | 0.135 | 34.5 | 63.4 | 0 | gompertz | 99.8 |
| 2 Rep 3 | Bristol | 0.153 | 32.3 | 69.8 | 0 | gompertz | 99.9 |
| 2 Rep 4 | Bristol | 0.167 | 34.3 | 51.6 | 0 | gompertz | 99.9 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 Rep 1 | Gazelle | 0.199 | 23.4 | 83.7 | 0 | gompertz | 99.6 |
| 2 Rep 2 | Gazelle | 0.233 | 23.7 | 82.6 | 0 | gompertz | 99.7 |
| 2 Rep 3 | Gazelle | 0.189 | 23.5 | 79.0 | 0 | gompertz | 99.7 |
| 2 Rep 4 | Gazelle | 0.174 | 25.5 | 79.8 | 0 | gompertz | 99.8 |
| 2 Rep 1 | Libravo | 0.166 | 32.2 | 43.0 | 0 | gompertz | 99.7 |
| 2 Rep 2 | Libravo | 0.134 | 31.4 | 31.2 | 0 | gompertz | 99.3 |
| 2 Rep 3 | Libravo | 0.210 | 30.9 | 50.4 | 0 | gompertz | 99.9 |
| 2 Rep 4 | Libravo | 0.164 | 30.3 | 47.1 | 0 | gompertz | 99.5 |
| 2 Rep 1 | Martina | 0.141 | 33.4 | 62.8 | 0.375 | glogistic | 99.9 |
| 2 Rep 2 | Martina | 0.097 | 29.0 | 62.9 | 0 | gompertz | 99.7 |
| 2 Rep 3 | Martina | 0.113 | 27.5 | 61.1 | 0 | gompertz | 99.7 |
| 2 Rep 4 | Martina | 0.118 | 29.4 | 55.7 | 0 | gompertz | 99.9 |
| 2 Rep 1 | Rocket | 0.122 | 33.9 | 50.6 | 0.051 | glogistic | 99.8 |
| 2 Rep 2 | Rocket | 0.149 | 33.0 | 57.0 | 0 | gompertz | 99.8 |
| 2 Rep 3 | Rocket | 0.093 | 33.9 | 38.0 | 0 | gompertz | 99.7 |
| 2 Rep 4 | Rocket | 0.133 | 34.3 | 40.2 | 0.187 | glogistic | 99.9 |
| | | | | | | | |
| 3 Rep 1 | Askari | * | * | * | * | * | * |
| 3 Rep 2 | Askari | * | * | * | * | * | * |
| 3 Rep 3 | Askari | * | * | * | * | * | * |
| 3 Rep 4 | Askari | * | * | * | * | * | * |
| 3 Rep 1 | Bristol | 0.062 | 43.3 | 63.1 | 0 | gompertz | 97.6 |
| 3 Rep 2 | Bristol | 0.122 | 34.6 | 74.1 | 0 | gompertz | 97.5 |
| 3 Rep 3 | Bristol | 0.081 | 38.9 | 75.2 | 0 | gompertz | 97.6 |
| 3 Rep 4 | Bristol | 0.232 | 33.2 | 52.1 | 0 | gompertz | 99.2 |
| 3 Rep 1 | Gazelle | 0.510 | 28.6 | 27.9 | 0 | gompertz | 99.8 |
| 3 Rep 2 | Gazelle | 0.530 | 28.0 | 29.0 | 0 | gompertz | 99.6 |
| 3 Rep 3 | Gazelle | 1.648 | 28.5 | 32.0 | 1.421 | glogistic | 98.9 |
| 3 Rep 4 | Gazelle | 1.007 | 27.7 | 36.6 | 0.011 | glogistic | 99.5 |
| 3 Rep 1 | Libravo | 0.383 | 31.8 | 27.6 | 0 | gompertz | 98.9 |
| 3 Rep 2 | Libravo | 0.330 | 30.9 | 31.8 | 0 | gompertz | 98.5 |
| 3 Rep 3 | Libravo | 0.288 | 32.2 | 32.6 | 0 | gompertz | 98.6 |
| 3 Rep 4 | Libravo | 0.294 | 31.5 | 22.1 | 0 | gompertz | 97.4 |
| 3 Rep 1 | Martina | 0.147 | 31.5 | 28.1 | 0 | gompertz | 96.8 |
| 3 Rep 2 | Martina | 0.096 | 34.4 | 44.2 | 0 | gompertz | 96.9 |
| 3 Rep 3 | Martina | 0.187 | 31.8 | 37.8 | 0 | gompertz | 97.2 |
| 3 Rep 4 | Martina | 0.111 | 31.7 | 34.6 | 0 | gompertz | 95.5 |
| 3 Rep 1 | Rocket | 0.153 | 33.6 | 33.2 | 0 | gompertz | 98.9 |
| 3 Rep 2 | Rocket | 0.117 | 35.6 | 28.7 | 0 | gompertz | 98.2 |
| 3 Rep 3 | Rocket | 0.130 | 33.2 | 37.8 | 0 | gompertz | 95.7 |
| 3 Rep 4 | Rocket | 0.132 | 32.5 | 29.2 | 0 | gompertz | 96.7 |

As in the laboratory germination test, the model tried to fit the generalised logistic function, but where it failed to converge due to $t$ approaching 0, the gompertz function was then fitted. Variance accounted for was over 99% in 50 out of 68 data sets (Table 5.3).

The majority of the curves were of the gompertz type, 57 out of 68, indicating the maximum emergence rate occurring in the early percentiles. The remaining 11 sets were of the generalised logistic curves, of which five were very close to the gompertz where $t = 0.011$. There were no curves for Askari in the third sowing due to very poor emergence figures. The early emergers in sowing one and two, as indicated by timepoint *m*, were Gazelle and Martina, closely followed by Libravo. In the third sowing, which had the lowest average temperature for emergence; early emergers were Gazelle, Libravo and Martina. Libravo appeared to quicken its time for emergence with the reduction in temperature that occurred with each successive sowing. Data are fitted curves for all varieties and sowings are compared in Figures 5.2 to 5.7.

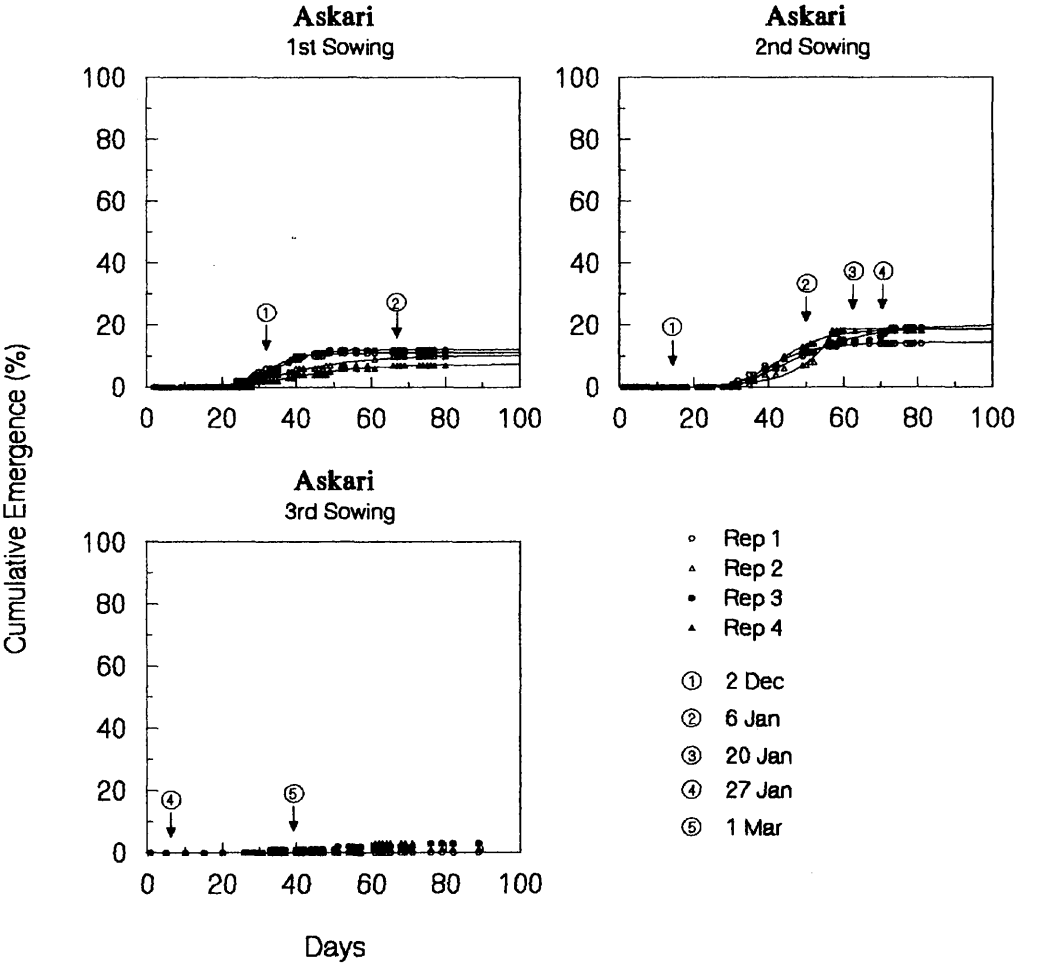



**Figure 5.2 Cumulative emergence curves of cv. Askari**

Askari (Figure 5.2) was the poorest performer in emergence, possibly due to deterioration of seed. All four replicates followed a similar pattern in each sowing. Emergence was highest in the second sowing, in contrast to the other cultivars, because the cold spell around 2 December occurred when emergence in the first sowing was only at 5%, thereby slowing and stopping the process.

The second sowing had a longer period of growing temperatures after initial emergence before a cold period on around 6 January concluded events. There was very little emergence for the 3rd sowing due to low temperatures on 1 March.

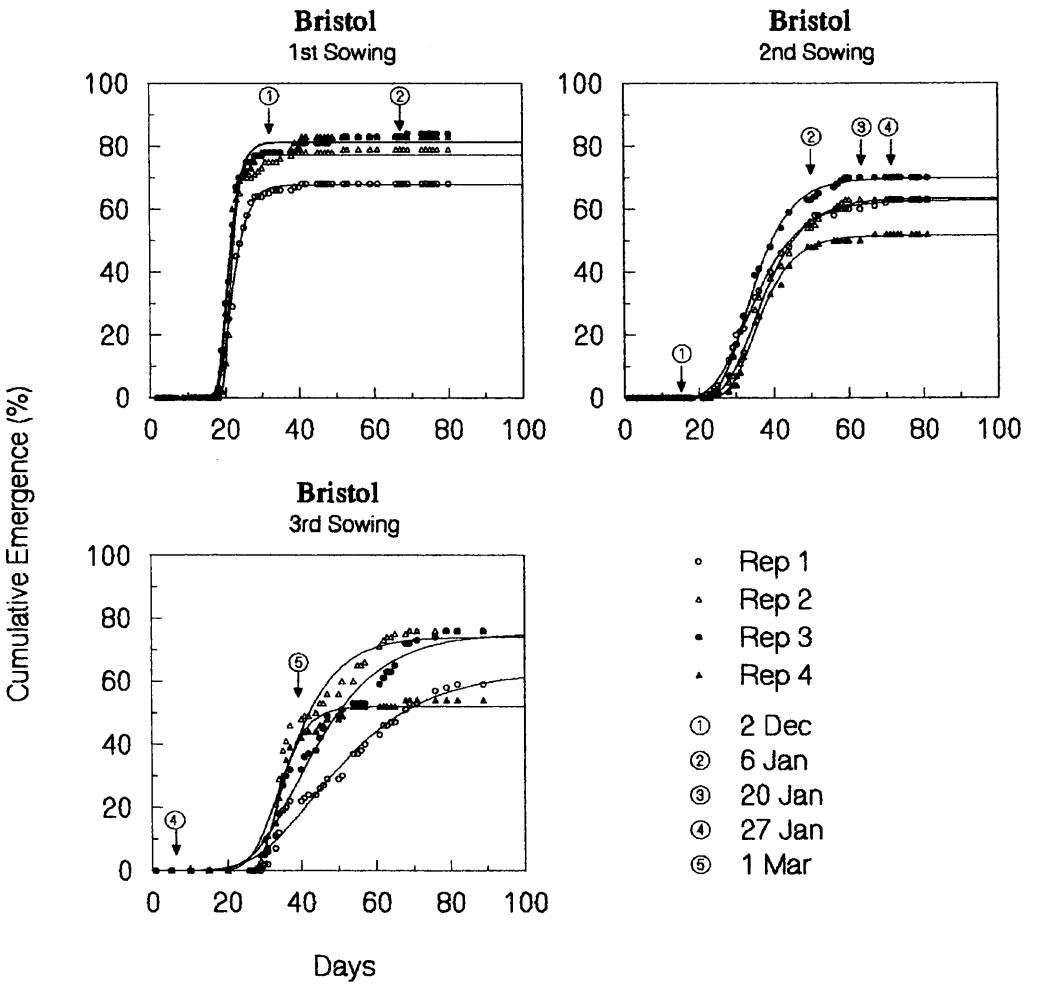



**Figure 5.3 Cumulative emergence curves of cv. Bristol**

For Bristol (Figure 5.3) the effect of the cold spell on 2 December appears to produce different responses between the first two sowings in that it stopped the

emergence in the first, but only reduced the emergence rate in the second sowing as it occurred before initial emergence. The cold periods of 6 January and 20 January respectively slowed and stopped the emergence of the second sowing. The third sowing showed a similar response in that the cold period of 1 March completely stopped replicate 4 but only reduced the emergence rate of the other replicates. After the cold period of around 1 March there were no other comparative periods of low temperatures.

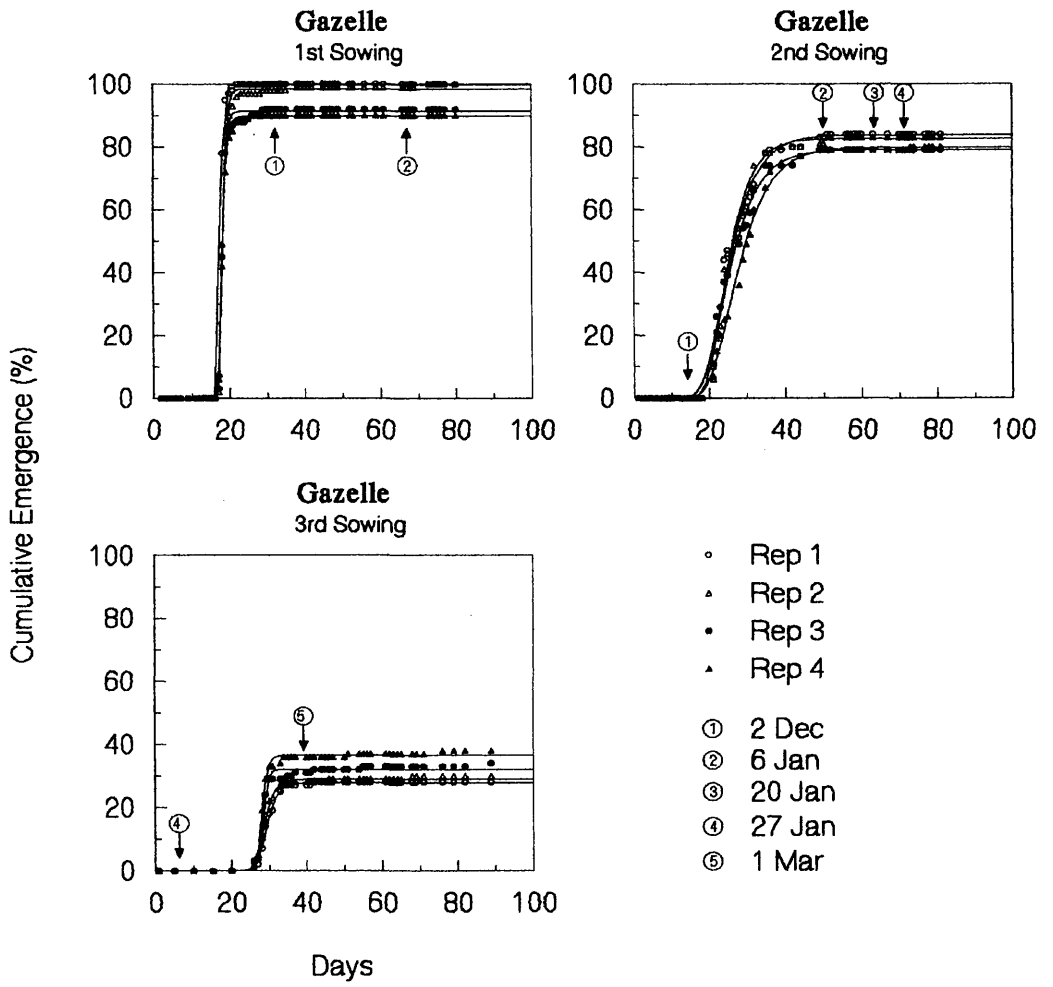



**Figure 5.4 Cumulative emergence curves of cv. Gazelle**

Gazelle (Figure 5.4) in the first sowing reached 90% emergence in all 4 replicates. This figure was reduced to approximately 80% in the second sowing where emergence rate was reduced, and the cessation of emergence appeared to be caused by

the cold spell occurring around 6 January. The third sowing in comparison only produced emergence around 30% as the cold period of 1 March occurred early in emergence.

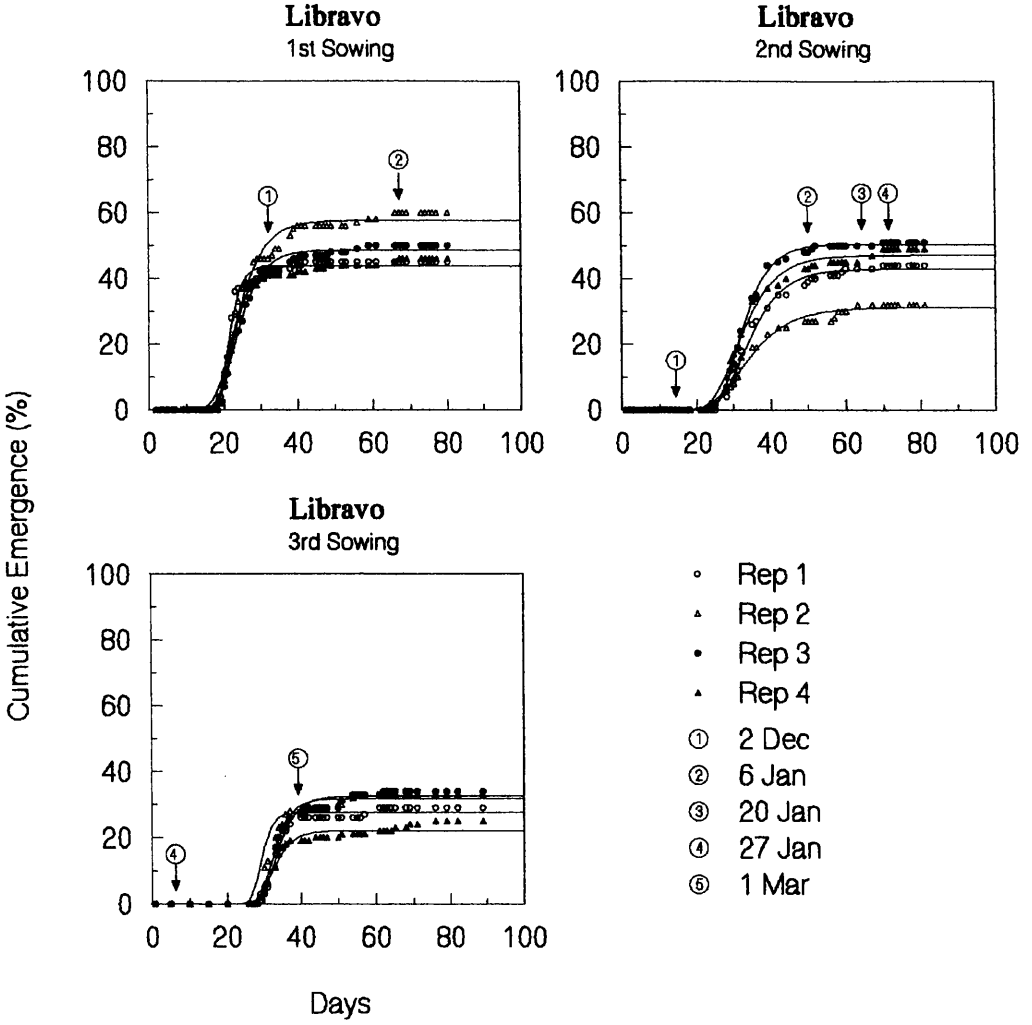



**Figure 5.5 Cumulative emergence curves of cv. Libravo**

In Libravo (Figure 5.5) the time to initial emergence was slightly longer than in most of the cultivars. Therefore emergence was stopped between 40% and 50% when the cold period of 2 December arrived. The relative pattern in the third sowing was similar in that a cold period on 1 March stopped emergence. There was variation in low temperature responses between replicates for the three sowings, some stopping immediately and others slowing down and then stopping on meeting another cold period.

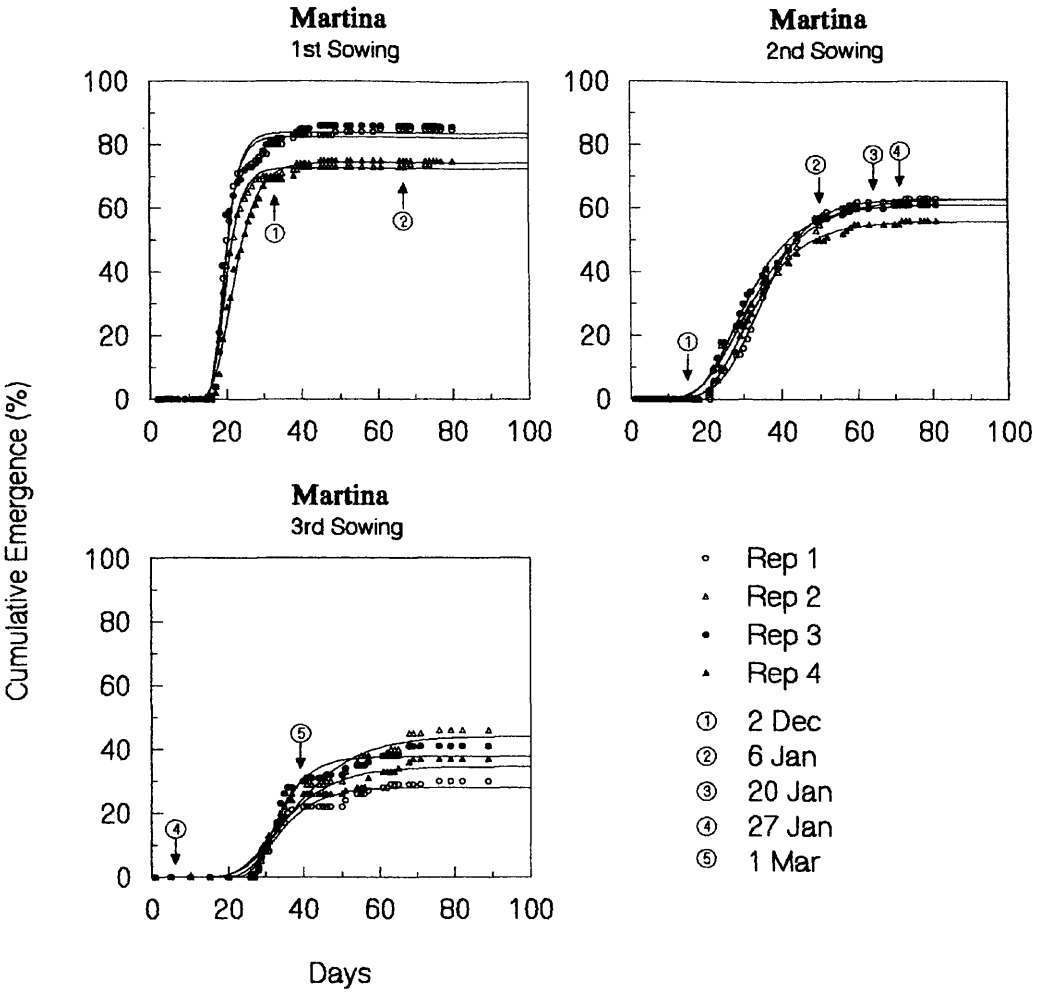



**Figure 5.6 Cumulative emergence curves of cv. Martina**

The cold weather period on 2 December also stopped emergence in the first sowing of Martina (Figure 5.6), but the reduction in emergence rate in the second sowing was more conspicuous than for the other cultivars. Periods of low temperature stopped emergence in all three sowings, not abruptly but gradually, particularly in the case of the third sowing where 10% emergence still occurred after the low temperatures around 1 March. There seemed to be a greater similarity between replicates in all three sowings in their responses to low temperature than in the other cultivars.

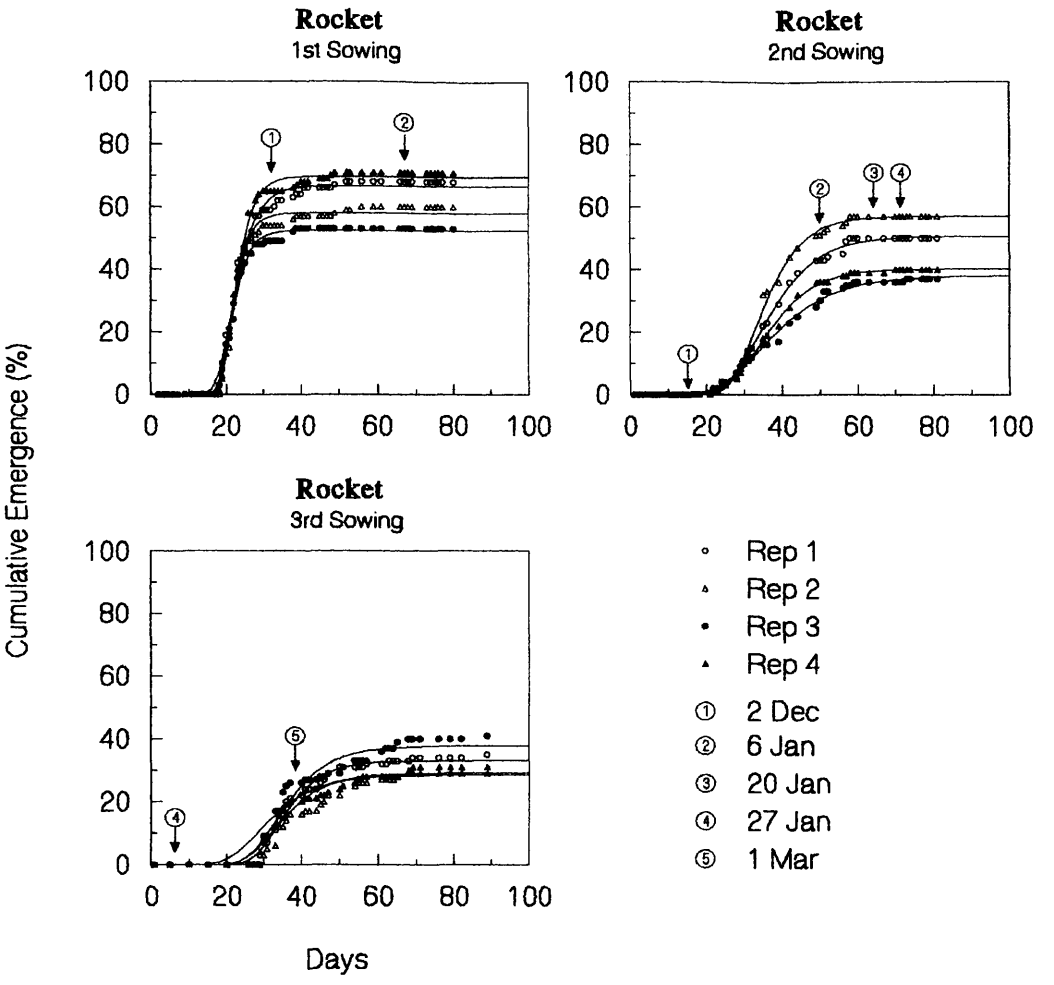



**Figure 5.7 Cumulative emergence curves of cv. Rocket**

The cold temperature response on 2 December for Rocket (Figure 5.7) again produced cessation of emergence in the first sowing and reduction of emergence rate in the second sowing. There was a greater spread of emergence between replicates during the period from 2 December to 6 January in the second sowing indicating variation in loss of emergence rate due to low temperature. Like Martina, there was no abrupt stoppage of emergence but a gradual decline.

## 5.4 Variation of rate of emergence with temperature

The relation between temperature and germination rate was examined in the laboratory germination test.

The defining equation is:

$$1/t = a(b^{(T-T_b)}-1)$$

where $T_b$ is the temperature at which $1/t$ is zero. In all the cultivars, except Bristol, the best fit model for each cultivar had a single common base temperature and parameter $b$ irrespective of percentile; only parameter $a$ varied between the percentiles. The temperature range in the field work was too narrow to enable an adequate fitting procedure. Therefore, seeds were assumed to have the same $T_b$ and $b$ as estimated in the laboratory germination test. The estimated time to germination $t_g$ for each cultivar was first calculated and compared to the time to emergence $t_e$. In the case of Bristol where parameter $b$ varied due to effects of non-germination in higher percentiles at low constant temperature, the figure for the 10th percentile was used. Average figures were obtained from data sets (Appendix 4) for the 10th, 20th and 50th percentiles (Table 5.4).

**Table 5.4 Comparison of estimated time to germination and time to emergence ( $T_b$ and $b$ taken from chapter 3)**

| | | | Ratio ($tg/te$) of time to germination/ time to emergence | | | |
|---|---|---|---|---|---|---|
| | Tb | b | 10% | 20% | 50% | Average |
| Askari | -1.4 | 1.107 | * | * | * | * |
| Bristol | -0.9 | 1.154 | 2.4 | 1.8 | 1.4 | 1.9 |
| Gazelle | -2.0 | 1.099 | 3.4 | 3.4 | 3.1 | 3.3 |
| Libravo | -0.6 | 1.078 | 5.0 | 5.1 | 5.3 | 5.1 |
| Martina | -1.8 | 1.096 | 4.1 | 4.2 | 4.2 | 4.2 |
| Rocket | 0.2 | 1.085 | 4.5 | 4.8 | 5.0 | 4.8 |

The reason for the differences in $t_g$ and $t_e$ is that it takes time for the radicle of the seed to penetrate the 2 cm layer of soil and open its dicotlyedon leaves. However the ratios of estimated time to germination and time to emergence varied between cultivars ranging from an average of 1.9 in Bristol to 5.1 in Libravo. The low ratio in Bristol might have been due to different responses to constant temperature, as used in the laboratory, and to the fluctuating temperatures that occurred in the field.

Next, curves were constructed using the values of $T_b$ and parameter $b$ measured in the laboratory and an estimation was made for the parameter $a$ in the field

(Table 5.5). The difference between $t_g$ and $t_e$ and result in percentiles in the field having a smaller parameter *a*.

**Table 5.5 Estimation of parameter *a* for each cultivar using a common $T_b$ and parameter *b***

| | 10% | 20% | 50% | 80% | 90% |
|---|---|---|---|---|---|
| Askari | 0.023 | * | * | * | * |
| Bristol | 0.024 | 0.022 | 0.020 | 0.015 | * |
| Gazelle | 0.045 | 0.042 | 0.039 | 0.036 | 0.033 |
| Libravo | 0.061 | 0.055 | 0.045 | * | * |
| Martina | 0.046 | 0.042 | 0.035 | 0.028 | * |
| Rocket | 0.067 | 0.061 | 0.050 | * | * |

Finally, the fitted curves are shown with data in Figure 5.8. Except for Askari, which had poor emergence, the range between percentiles is indicative of the within-cultivar heterogeneity: Martina being most heterogeneous (between 10th and 50th percentile), followed by in decreasing order Libravo, Rocket, Bristol and Gazelle (see also Table 5.5).

To complete the analysis of pre-winter emergence, thermal weighting of the emergence of each sowing was made. The equation that describes the relationship between temperature and rate of germination could now be applied to emergence:

$$\text{emergence} = a_i(b^{(T-T_b)}-1)$$

This equation can be transposed so that we find *1/a*. Using the actual average soil temperatures, calculated daily, combined with the $T_b$ and parameter *b* values used previously for each cultivar, *1/a* can be derived for each observation of emergence. In order to preserve clarity only replicate 1 was used for the inter-cultivar comparison of thermally weighted time.

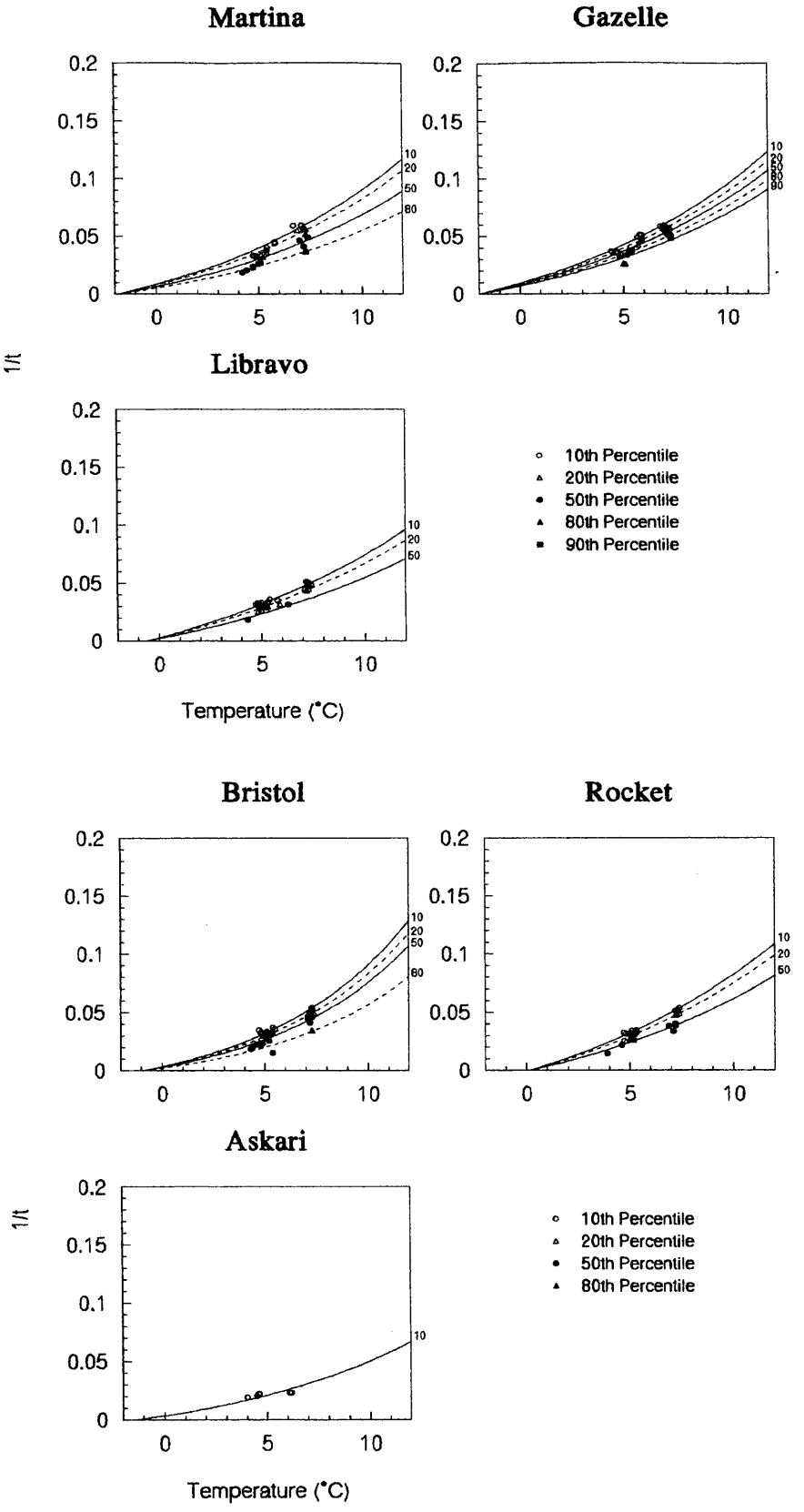



**Figure 5.8 Rate of emergence with temperature for all cultivars**

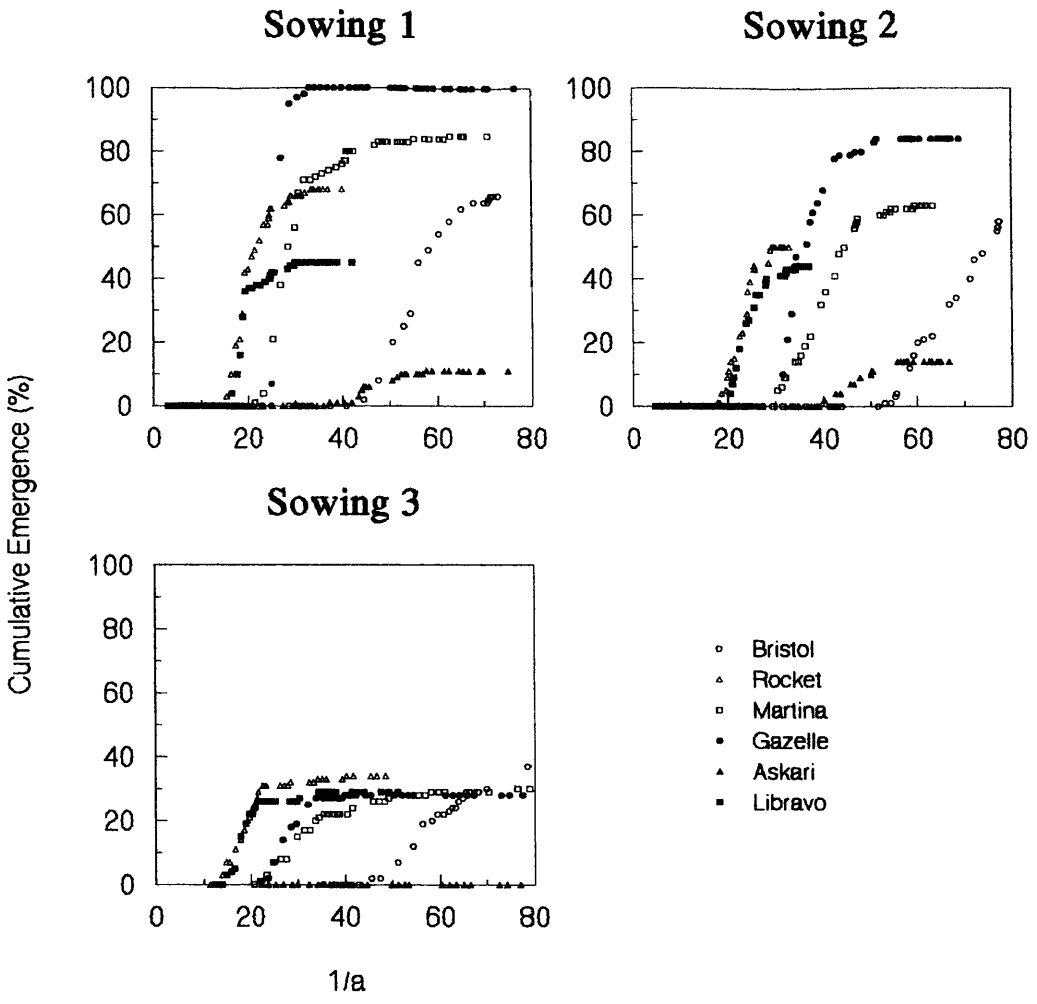



**Figure 5.9 Cumulative emergence in relation to thermally weighted time for all sowings, replicate 1 only as an example**

In the plots of emergence against thermally weighted time (Figure 5.9) the cultivars formed three distinct groupings of similar *1/a*; these were Libravo and Rocket, Martina and Gazelle, Bristol and Askari. These groups maintain their rankings despite variation between sowings, in the absolute value of emergence on *1/a*. Such variation, however, shows the influence of factors other than current temperature. In the plots of sowing 2, for instance, the cultivars are exhibiting delayed emergence and slower rate, with Gazelle, Martina and Bristol being the worst affected, possibly as a result of the cold spell around 2 December.

## 5.5 Post-winter emergence

The experiment was monitored until the end of July 1998 to observe any post-winter emergence (Table 5.6) as emergence during this period could put winter type oilseed rape in flowering synchrony with spring-sown oilseed rape.

**Table 5.6 Post-winter emergence (%) of field trials**

| 1st Sowing | Rep 1 | Rep 2 | Rep 3 | Rep 4 |
|---|---|---|---|---|
| Askari | 0 | 1 | 1 | 0 |
| Bristol | 0 | 0 | 0 | 0 |
| Gazelle | 0 | 0 | 0 | 0 |
| Libravo | 0 | 1 | 0 | 0 |
| Martina | 0 | 3 | 1 | 0 |
| Rocket | 0 | 2 | 0 | 2 |
| 2nd Sowing | Rep 1 | Rep 2 | Rep 3 | Rep 4 |
| Askari | 2 | 2 | 2 | 2 |
| Bristol | 1 | 1 | 3 | 0 |
| Gazelle | 0 | 0 | 0 | 0 |
| Libravo | 4 | 3 | 0 | 2 |
| Martina | 8 | 3 | 5 | 0 |
| Rocket | 2 | 3 | 1 | 2 |
| 3rd Sowing | Rep 1 | Rep 2 | Rep 3 | Rep 4 |
| Askari | 0 | 0 | 0 | 0 |
| Bristol | 0 | 0 | 0 | 0 |
| Gazelle | 0 | 0 | 0 | 0 |
| Libravo | 0 | 0 | 0 | 0 |
| Martina | 0 | 0 | 0 | 0 |
| Rocket | 0 | 0 | 0 | 0 |

Post-winter emergence only occurred in the first two sowings. It was possible that dormant seeds in the third sowing could emerge later than the completion date of the experiment, but then it would be in synchrony with the normal life cycle of winter type oilseed rape.

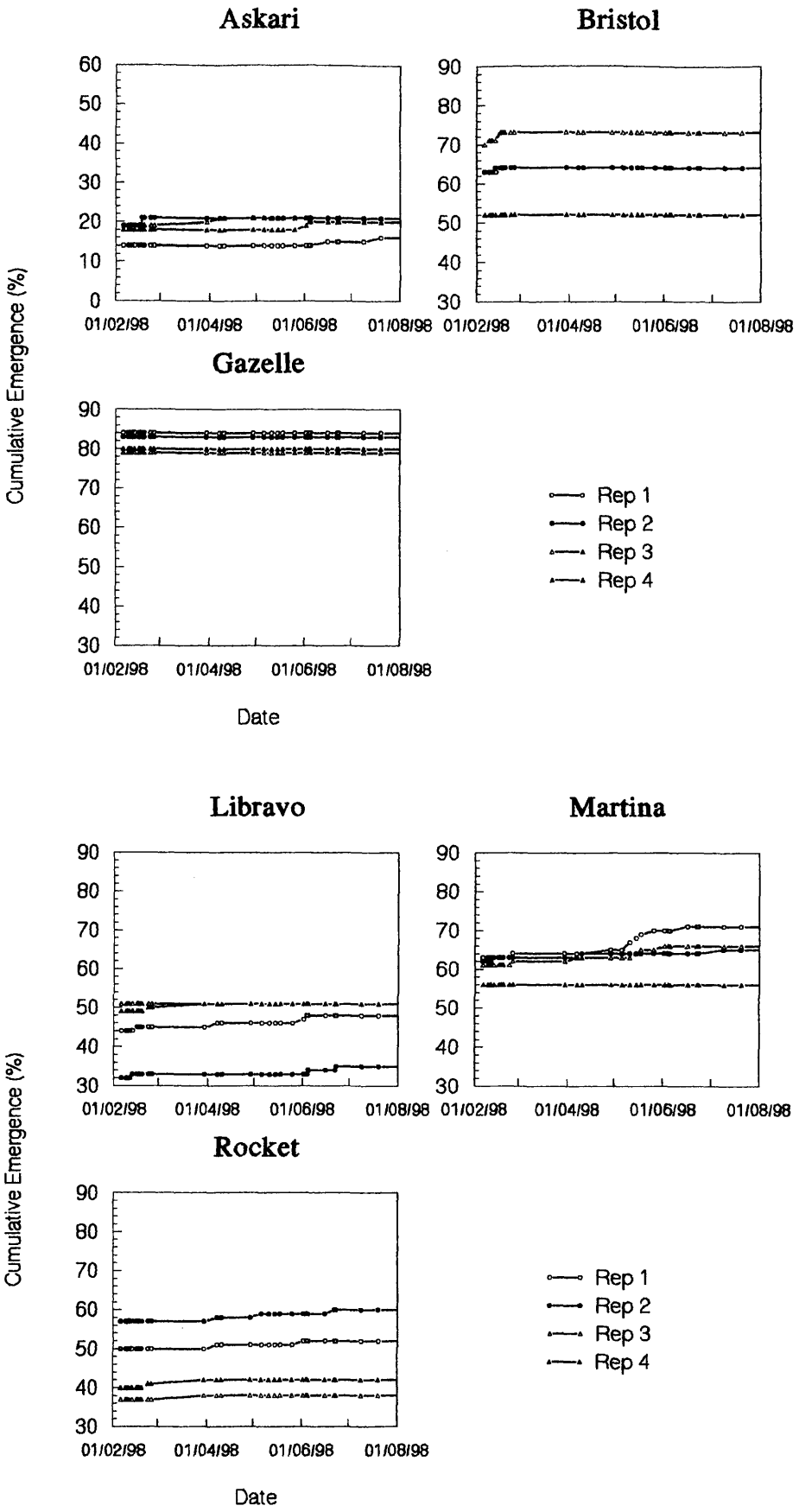



**Figure 5.10 Post-winter emergence (sowing 2) for all cultivars**

There was a greater emergence in sowing two than in sowing one, with Martina having the highest percentage in both the sowings and Gazelle not having any post-winter emergence. In sowing one Martina and Rocket were the only cultivars that had more than a single plant emerging. In sowing two every cultivar, with the exception of Gazelle, had more than one plant emerging in at least one replicate. Both sowings had variance between replicates, with replicate two having the highest emergence in sowing one and replicate one having the highest in sowing two.

In sowing two (Figure 5.10) emergence for the cultivar Bristol was limited to a warm phase in mid February only, whereas Askari had emergence not only in that same period, but also the start of April, and in June and July.

Post-winter emergence occurred in Libravo, Martina and Rocket (Figure 5.10) in mid February during a prolonged mild spell. A cold period at the start of March meant there was no emergence for that month, continuing climbing temperatures during March resulted in emergence for all three cultivars at the start of April.

The next three months of May, June and July also saw emergence in all three cultivars, but whilst there were only individual plants emerging per month for Rocket and Libravo, the month of May provided 4% emergence for one replicate of Martina. Martina was the only cultivar to have emergence in the month of July.

## 5.6 Discussion

The principal factor, in evidence, on the effects of percentage emergence, emergence rate and secondary dormancy was the timing, duration and intensity of periods of low temperature.

The poor performance of Askari in all three sowings means that it cannot be taken into account for cultivar comparison. The best representative examples for cultivar comparison in field performance are Gazelle and Martina, the extremes of within-cultivar heterogeneity, all the other cultivars lying midway between. In all three sowings Gazelle had an overall faster emergence rate than Martina, but the variation in the emergence rate between percentiles was greatest in Martina. Gazelle achieved the

highest emergence in the first two sowings, but in the third sowing was less than Martina.

There were differences between cultivar replicates in all the sowings. There was no replicate having a predominance of having either the highest or lowest emergence figure ruling out positional bias. There was also no evidence that difference in emergence for replicates were accounted for, either by cultivar or sowing. The differences in soil temperatures between replicates were very slight and these differences did not correspond to differences in emergence between replicates. The conclusion is that the differences in emergence between replicates are possibly due to standard sampling error.

The results reiterated previous findings in over-wintering emergence of oilseed rape (Squire, 1999). The profiles of the time to emergence curves were similar in appearance to the time to germination curves from the laboratory germination test; both sets have predominantly gompertz type curves indicating non-linearity in the early percentiles at lower temperatures. Variation in estimated parameter *a* between percentiles was evident, indicating heterogeneity among cultivars. The ranking of the cultivars from the most heterogeneous to the least, denoted by the variation in parameter *a,* was the same ranking as in chapter 4 indicating for the first time a strong association between genotypic variation and phenotypic variation in emergence.

Low temperatures can induce secondary dormancy in some cultivars (Marshall & Squire, 1996). In chapter 3, this occurred in the laboratory germination test, where there was variation in the application of temperature stimuli to break dormancy. Post-winter emergence signifies the breaking of secondary dormancy with rising temperatures and a higher daily amplitude (Squire, 1999). The cultivar Martina showed the greatest post-winter emergence spread over the longest time period whilst Gazelle showed no post-winter emergence. The ranking order in decreasing heterogeneity was the same ranking order for pre-winter emergence and genetic heterogeneity confirming the association between variation in germination traits and variation in genotype.

# Chapter 6

# CONCLUSION AND FURTHER WORK

## 6.1 Principal findings

The principal findings of this work were:

- Phenotypic variation in temperature-related germination traits was quantified by the parameter of simple curves.
- The exponential relation between rate (1/time) and temperature found for one cultivar previously, was confirmed by all six cultivars.
- Cultivars differed considerably in their susceptibility to induction of secondary dormancy at low temperature.
- Cultivars also varied in temperature stimuli required for dormancy break.
- Cultivars could be clearly ranked according to their phenotypic heterogeneity.
- Inter-cultivar variation in genetic heterogeneity was found using SSR primers.
- A relation between variability in markers and variability in germination was identified.
- Strong association of specific phenotypes with genotype was found in Martina but not the other cultivars.
- Emergence in the field, rate of emergence and secondary dormancy depended particularly on timing, duration and intensity of low temperature below 2°C in the field.
- A strong similarity was detected between laboratory germination and field emergence curves.
- Phenotypic heterogeneity among cultivars of rate of emergence and post-winter emergence were also consistent with genetic heterogeneity among cultivars.

## 6.2 Heterogeneity in germination rate among cultivars

The laboratory experiment revealed a substantial inter- and intra- cultivar variation in germination traits. Previous germination work had found non-linearity and

non-germination at low temperature for the cultivar Martina (Marshall & Squire, 1996). Exponential models of temperature-rate relations indicated parameter *a* was the main variable in the rate of germination (Squire *et al*, 1997a). This work not only reiterated theses findings but also established collectively the parameters and traits that best distinguish inter-cultivar variation in terms of their heterogeneity. These criteria are:

- The variation in parameter *a* between percentiles.
- The size of the non-germinating fraction at low temperature.
- The temperature stimuli required in breaking dormancy.

The field experiment confirmed previous work that emergence was affected when temperature falls below 10°C (Squire, 1999). Similarity was evident between the time to germination and time to emergence curves, both indicating non-linearity at low temperatures. The exponential curves for temperature-rate relations from the laboratory, with an estimated reduced parameter *a* to allow for time differences between germination and emergence, were used in conjunction with emergence data for laboratory and field comparison (Squire, 1999). The close fits between the emergence data and germination curves indicated the pertinence of using laboratory models for application in the field. The ranking of the cultivars in terms of their heterogeneity, described by the variation in parameter *a* between percentiles, slightly changed from laboratory to field with Martina being more heterogeneous than Libravo. The variation between cultivars was less pronounced in emergence compared to germination due to above average autumnal temperatures.

Post-winter emergence produced the greatest inter-cultivar variation in the field, the ranking in decreasing heterogeneity being the same for emergence rate, thereby verifying the criteria defined in the laboratory.

These findings confirms previous work (Pekrun *et al.*, 1997; Marshall & Squire, 1996; Squire *et al.*, 1997a) that oilseed rape cultivars contain much heterogeneity in germination rate and secondary dormancy, even cultivars that have

passed through the national list trials contain variability when stressed by low temperatures. At present, the cultivar Martina has not been fully accepted by NIAB (National Institute of Agricultural Botany) because of the known variability. All the others have, however. In order to ascertain whether there is a hidden variability in germination, standard seed testing must be carried out at low temperatures in addition to the other set requirements.

## 6.3 Ecological implications

The detection of substantial variability in germination and dormancy in cultivars has important implications for the origin of feral oilseed rape seedbanks. It is proposed that cultivars having great variability in germination have a greater chance of becoming a successful feral plant. A cultivar's survival, when faced with adverse weather conditions and competition from other plants, increases with a broader germination time. It also increases the time window for inter-cultivar hybridization with feral types and also allows the plant to emerge outside the seasonal agricultural practices for weed prevention.

There is at present, no knowledge whether variability in germination leads to longer term persistence in the soil. More generally however, seeds which a greater inherent dormancy as in the case of *Brassica rapa* (Landbo & Jørgensen, 1997), whether primary or secondary, survive for longer periods in the soil and also contribute to longetivity of regenerative seedbanks (Sagar & Mortimer, 1976).

The ranking order of genetic variation was the same as the ranking order from the field emergence trials indicating a genetic basis for phenotypic heterogeneity. However, the association between genotype and phenotype was strongly evident in Martina only. A much more extensive study using many primers would probably be required to test rigorously for a specific association between germination and dormancy traits, and markers. The findings of the DNA profiling is that pure seed such as Gazelle will probably have much less feral genetic material than the variable cultivars such as Martina and Libravo. Plant breeders should be more cautious about

introducing variability when breeding new lines and ensure that cultivars meet the EEC requirements for distinctiveness, uniformity and stability. Testing seed for uniformity is normally done at temperatures much higher than those that induce secondary dormancy. Perhaps therefore a change in testing procedure is necessary. The application of these results would be especially pertinent when considering the use of genetically modified oilseed rape where the prevention of transgene movement to the environment may be important.

## 6.4 Further work

The results of this study have indicated that the following further line of research would be profitable.

In order to fully assess the link between secondary dormancy and potential persistence, creation of controlled artificial seedbanks containing both uniform and variable cultivars could be constructed for ecological monitoring over several years.

Further work in exploring the association between genetic base and variation in germination could be achieved by examining phenotypic/genotypic interactions of progeny from self-pollinated phenotypes. In the case of variable cultivars such as Martina, where there were two distinct populations, a greater divergence in germination traits should occur between genotypes. In order to study the maternal/paternal interactions involved in germination, experiments on plant crosses of known germination traits and DNA profiles, can give indications to the effect of hybridization. Self-pollination of these hybrids with further investigations into their phenotype/genotype association could aid plant breeders in screening potential new varieties for undesirable germination characteristics.

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## APPENDIX 1

### Data logger Program : Thermal Plate 1996

| | | | |
|---|---|---|---|
| *1 | Table 1 Programs | | |
| 01: 60 | Sec. Execution Interval | | |
| **01**: P17 | Panel Temperature | **02**: P10 | Battery Voltage |
| 01: 17 | Loc [:Panel] | 01: 18 | Loc [:Batt V] |
| **03**: P13 | Thermocouple Temp (SE) | **04**: P92 | If time is |
| 01: 16 | Reps | 01: 0 | minutes into a |
| 02: 1 | 5 mV slow Range | 02: 60 | minute interval |
| 03: 1 | IN Chan | 03: 10 | Set flag 0 (out) |
| 04: 3 | Type K (Chromel-Alumel) | | |
| 05: 17 | Ref Temp Loc Panel | | |
| 06: 1 | Loc [:Temp] | | |
| 07: 1 | Mult | | |
| 08: 0.0000 | Offset | | |
| **05**: P77 | Real Time | **06**: P71 | Average |
| 01: 110 | Day,Hour-Minute | 01: 16 | Reps |
| | | 02: 1 | Loc Temp |
| **07**: P71 | Average | **08**: P73 | Maximum |
| 01: 16 | Reps | 01: 16 | Reps |
| 02: 1 | Loc Temp | 02: 1 | Loc Temp |
| **09**: P74 | Minimum | **10**: P | End Table 1 |
| 01: 16 | Reps | | |
| 02: 1 | Loc Temp | | |

## APPENDIX 2

### Data logger Program : Field Trials 1997

| | | | |
|---|---|---|---|
| *1 | Table 1 Programs | | |
| 01: 600 | Sec. Execution Interval | | |
| **01**: P10 | Battery Voltage | **02**: P10 | Panel Temperature |
| 01: 2 | Loc [:Batt V] | 01: 1 | Loc [:Panel] |
| **03**: P86 | Do | **04**: P87 | Beginning of Loop |
| 01: 41 | Set high Port 1 | 01: 0 | Delay |
| | | 02: 14 | Loop Count |
| **05**: P22 | Excitation with Delay | **06**: P14 | T.C. Temp (DIFF) |
| 01: 1 | EX Chan | 01: 1 | Rep |
| 02: 1 | Delay w/EX (units=.01sec) | 02: 1 | 5 mV slow Range |
| 03: 1 | Delay after EX (units=.01sec) | 03: 1 | IN Chan |
| 04: 5000 | mV Excitation | 04: 3 | Type K (Chromel-Alumel) |
| **06**: P14 | Thermocouple Temp (DIFF) | **07**: P14 | T.C. Temp (DIFF) |
| 01: 1 | Rep | 01: 1 | Rep |
| 02: 1 | 5 mV slow Range | 02: 1 | 5 mV slow Range |
| 03: 1 | IN Chan | 03: 2 | IN Chan |
| 04: 3 | Type K (Chromel-Alumel) | 04: 3 | Type K (Chromel-Alumel) |
| 05: 1 | Ref Temp Loc | 05: 1 | Ref Temp Loc |
| 06: 3-- | Loc : Temp#1 - Rep 1,2 | 06: 17-- | Loc : Temp#15 -Rep 3,4 |
| 07: 1 | Mult | 07: 1 | Mult |
| 08: 0 | Offset | 08: 0 | Offset |

| | | | |
|---|---|---|---|
| **08**: P95 | End | **09**: P86 | Do |
| | | 01: 51 | Set low Port 1 |
| **10**: P86 | Do | **11**: P87 | Beginning of Loop |
| 01: 42 | Set high Port 2 | 01: 0000 | Delay |
| | | 02: 7 | Loop Count |
| **12**: P22 | Excitation with Delay | **13**: P14 | T.C. Temp (DIFF) |
| 01: 2 | EX Chan | 01: 1 | Rep |
| 02: 1 | Delay w/EX (units=.01sec) | 02: 1 | 5 mV slow Range |
| 03: 1 | Delay after EX (units=.01sec) | 03: 3 | IN Chan |
| 04: 5000 | mV Excitation | 04: 3 | Type K (Chromel-Alumel) |
| | | 05: 1 | Ref Temp Loc |
| | | 06: 31-- | Loc : Temp#29-Row 1,3 |
| | | 07: 1 | Mult |
| | | 08: 0 | Offset |
| **14**: P14 | Thermocouple Temp (DIFF) | **15**: P95 | End |
| 01: 1 | Rep | | |
| 02: 1 | 5 mV slow Range | | |
| 03: 4 | IN Chan | | |
| 04: 3 | Type K (Chromel-Alumel) | | |
| 05: 1 | Ref Temp Loc | | |
| 06: 38-- | Loc : Temp#36 Row 2 & 4 | | |
| 07: 1 | Mult | | |
| 08: 0 | Offset | | |

| | | | |
|---|---|---|---|
| **16**: P86 | Do | **17**: P92 | If time is |
| 01: 52 | Set low Port 2 | 01: 0 | minutes into a |

| | | | |
|---|---|---|---|
| | | 02: 60 | minute interval |
| | | 03: 10 | Set high Flag 0 (output) |
| **18**: P80 | Set Storage Area | **19**: P77 | Real Time |
| 01: 1 | Final Storage Area | 01: 110 | Day,Hour-Minute |
| 02: 1 | Array ID or location | | |
| **20**: P71 | Average | **21**: P92 | If time is |
| 01: 42 | Reps | 01: 1430 | minutes into a |
| 02: 3 | Loc | 02: 1440 | minute interval |
| | | 03: 10 | Set high Flag 0 (output) |
| **22**: P80 | Set Storage Area | **23**: P73 | Maximize |
| 01: 1 | Final Storage Area | 01: 44 | Reps |
| 02: 24 | Array ID or location | 02: 0 | Value only |
| | | 03: 1 | Loc |
| **24**: P74 | Minimize | **25**: P96 | Serial Output |
| 01: 44 | Reps | 01: 30 | SM192/716/CSM1 |
| 02: 0 | Value only | | |
| 03: 1 | Loc | | |
| **26**: P | End Table 1 | ***4** | Mode 4 Output Options |
| | | 01: 00 | Tape/Printer Option |
| | | 02: 00 | Printer Baud Option |
| ***A** | Mode 10 Memory Allocation | | |
| 01: 45 | Input Locations | | |
| 02: 140 | Intermediate Locations | | |

## APPENDIX 3

### T.D.R. readings of soil moisture (%)

| Date | Sowing | Rep1 | Rep2 | Rep3 | Rep4 |
|---|---|---|---|---|---|
| 7/11/97 | 1 | 29 | 28 | 33 | 23 |
| 21/11/97 | 1 | 33 | 29 | 35 | 32 |
| | 2 | 25 | 27 | 24 | 31 |
| 22/12/97 | 1 | 30 | 27 | 30 | 29 |
| | 2 | 25 | 25 | 24 | 29 |
| 27/1/98 | 1 | 29 | 25 | 30 | 31 |
| | 2 | 25 | 22 | 26 | 29 |
| | 3 | 27 | 23 | 24 | 24 |
| 23/2/98 | 1 | 28 | 24 | 28 | 30 |
| | 2 | 25 | 22 | 26 | 29 |
| | 3 | 26 | 23 | 25 | 22 |
| 19/3/98 | 1 | 27 | 22 | 28 | 32 |
| | 2 | 29 | 22 | 28 | 29 |
| | 3 | 26 | 23 | 27 | 20 |

# APPENDIX 4

## Ratios of time to estimated germination/ time to emergence for all percentiles

Martina

| T10M | P10M | Gest | Ratio | T20M | P20M | Gest | Ratio | T50M | P50M | Gest | Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.1 | 0.059 | 0.219 | 3.7 | 7.3 | 0.056 | 0.205 | 3.7 | 7.4 | 0.049 | 0.172 | 3.5 |
| 6.7 | 0.059 | 0.205 | 3.5 | 6.9 | 0.055 | 0.192 | 3.5 | 7.0 | 0.046 | 0.161 | 3.5 |
| 7.1 | 0.060 | 0.219 | 3.7 | 7.2 | 0.056 | 0.201 | 3.6 | 7.4 | 0.049 | 0.172 | 3.5 |
| 7.2 | 0.057 | 0.223 | 3.9 | 7.3 | 0.052 | 0.205 | 4.0 | 7.2 | 0.041 | 0.167 | 4.1 |
| 5.4 | 0.038 | 0.163 | 4.3 | 5.2 | 0.032 | 0.141 | 4.4 | 4.2 | 0.019 | 0.095 | 5.1 |
| 5.8 | 0.044 | 0.175 | 4.0 | 5.4 | 0.036 | 0.147 | 4.0 | 4.7 | 0.023 | 0.106 | 4.7 |
| 5.8 | 0.045 | 0.175 | 3.9 | 5.3 | 0.038 | 0.144 | 3.8 | 4.7 | 0.024 | 0.106 | 4.4 |
| 5.4 | 0.040 | 0.163 | 4.0 | 5.1 | 0.034 | 0.139 | 4.0 | 4.4 | 0.021 | 0.099 | 4.8 |
| 4.9 | 0.032 | 0.148 | 4.6 | 4.9 | 0.026 | 0.133 | 5.2 | * | * | * | * |
| 4.8 | 0.033 | 0.145 | 4.4 | 5.1 | 0.027 | 0.139 | 5.1 | * | * | * | * |
| 4.9 | 0.033 | 0.148 | 4.5 | 5.1 | 0.029 | 0.139 | 4.7 | * | * | * | * |
| 4.7 | 0.034 | 0.142 | 4.2 | 5.0 | 0.027 | 0.136 | 5.0 | * | * | * | * |

Martina

| T80M | P80M | Gest | Ratio | T90M | P90M | Gest | Ratio |
|---|---|---|---|---|---|---|---|
| 7.3 | 0.037 | 0.136 | 3.7 | * | * | * | * |
| * | * | * | * | * | * | * | * |
| 7.3 | 0.038 | 0.136 | 3.6 | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |

Gazelle

| T10M | P10M | Gest | Ratio | T20M | P20M | Gest | Ratio | T50M | P50M | Gest | Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.0 | 0.059 | 0.199 | 3.4 | 7.0 | 0.059 | 0.186 | 3.2 | 7.2 | 0.057 | 0.166 | 2.9 |
| 6.8 | 0.058 | 0.193 | 3.3 | 7.0 | 0.057 | 0.186 | 3.3 | 7.0 | 0.055 | 0.161 | 2.9 |
| 7.0 | 0.059 | 0.199 | 3.4 | 7.0 | 0.057 | 0.186 | 3.2 | 7.2 | 0.055 | 0.166 | 3.0 |
| 7.0 | 0.059 | 0.199 | 3.4 | 7.0 | 0.058 | 0.186 | 3.2 | 7.2 | 0.055 | 0.166 | 3.0 |
| 5.9 | 0.051 | 0.165 | 3.2 | 5.9 | 0.046 | 0.154 | 3.3 | 5.4 | 0.037 | 0.121 | 3.2 |
| 5.7 | 0.049 | 0.159 | 3.3 | 5.8 | 0.045 | 0.151 | 3.4 | 5.3 | 0.038 | 0.119 | 3.2 |
| 5.8 | 0.051 | 0.162 | 3.2 | 5.9 | 0.046 | 0.154 | 3.4 | 5.4 | 0.036 | 0.121 | 3.3 |
| 5.9 | 0.047 | 0.165 | 3.5 | 5.7 | 0.042 | 0.149 | 3.5 | 5.2 | 0.033 | 0.117 | 3.5 |
| 4.7 | 0.035 | 0.131 | 3.7 | 4.8 | 0.033 | 0.125 | 3.8 | * | * | * | * |
| 4.6 | 0.036 | 0.129 | 3.6 | 4.8 | 0.033 | 0.125 | 3.7 | * | * | * | * |
| 4.7 | 0.036 | 0.131 | 3.7 | 4.9 | 0.035 | 0.128 | 3.7 | * | * | * | * |
| 4.4 | 0.037 | 0.124 | 3.4 | 4.5 | 0.035 | 0.118 | 3.3 | * | * | * | * |

Gazelle

| T80M | P80M | Gest | Ratio | T90M | P90M | Gest | Ratio |
|---|---|---|---|---|---|---|---|
| 7.2 | 0.055 | 0.141 | 2.6 | 7.2 | 0.054 | 0.126 | 2.3 |
| 7.1 | 0.052 | 0.139 | 2.6 | 7.2 | 0.051 | 0.126 | 2.5 |
| 7.3 | 0.052 | 0.143 | 2.7 | 7.3 | 0.048 | 0.128 | 2.6 |
| 7.3 | 0.051 | 0.143 | 2.8 | * | * | * | * |
| 5.1 | 0.026 | 0.097 | 3.8 | * | * | * | * |
| 5 | 0.026 | 0.096 | 3.7 | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |

Libravo

| T10M | P10M | Gest | Ratio | T20M | P20M | Gest | Ratio | T50M | P50M | Gest | Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.3 | 0.050 | 0.248 | 4.9 | 7.3 | 0.047 | 0.228 | 4.8 | * | * | * | * |
| 7.2 | 0.051 | 0.244 | 4.8 | 7.1 | 0.046 | 0.220 | 4.8 | 6.3 | 0.032 | 0.158 | 5.0 |
| 7.4 | 0.049 | 0.252 | 5.2 | 7.2 | 0.044 | 0.224 | 5.1 | * | * | * | * |
| 7.3 | 0.049 | 0.248 | 5.1 | 7.3 | 0.045 | 0.228 | 5.1 | * | * | * | * |
| 5.3 | 0.033 | 0.171 | 5.1 | 5.3 | 0.030 | 0.157 | 5.3 | * | * | * | * |
| 5.0 | 0.033 | 0.160 | 4.9 | 5.0 | 0.027 | 0.147 | 5.5 | * | * | * | * |
| 5.8 | 0.035 | 0.189 | 5.4 | 5.9 | 0.032 | 0.177 | 5.5 | 4.3 | 0.019 | 0.104 | 5.6 |
| 5.4 | 0.036 | 0.174 | 4.8 | 5.2 | 0.032 | 0.153 | 4.8 | * | * | * | * |
| 4.9 | 0.031 | 0.157 | 5.0 | 5.2 | 0.029 | 0.153 | 5.3 | * | * | * | * |
| 4.8 | 0.033 | 0.153 | 4.7 | 4.9 | 0.030 | 0.144 | 4.8 | * | * | * | * |
| 4.7 | 0.032 | 0.150 | 4.7 | 5.0 | 0.029 | 0.147 | 5.1 | * | * | * | * |
| 4.8 | 0.031 | 0.153 | 4.9 | 4.8 | 0.025 | 0.141 | 5.5 | * | * | * | * |

Libravo

| T80M | P80M | Gest | Ratio | T90M | P90M | Gest | Ratio |
|---|---|---|---|---|---|---|---|
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |

Bristol

| T10M | P10M | Gest | Ratio | T20M | P20M | Gest | Ratio | T50M | P50M | Gest | Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.3 | 0.052 | 0.132 | 2.5 | 7.4 | 0.049 | 0.091 | 1.9 | 7.2 | 0.041 | 0.053 | 1.3 |
| 7.2 | 0.050 | 0.129 | 2.6 | 7.1 | 0.049 | 0.085 | 1.8 | 7.1 | 0.045 | 0.052 | 1.2 |
| 7.3 | 0.054 | 0.132 | 2.4 | 7.3 | 0.051 | 0.089 | 1.7 | 7.3 | 0.046 | 0.055 | 1.2 |
| 7.3 | 0.053 | 0.132 | 2.5 | 7.3 | 0.051 | 0.089 | 1.8 | 7.3 | 0.046 | 0.055 | 1.2 |
| 5.4 | 0.037 | 0.086 | 2.3 | 5.3 | 0.032 | 0.054 | 1.7 | 4.8 | 0.023 | 0.027 | 1.2 |
| 5.1 | 0.033 | 0.080 | 2.4 | 5.1 | 0.030 | 0.052 | 1.7 | 4.6 | 0.022 | 0.026 | 1.2 |
| 5.5 | 0.036 | 0.089 | 2.5 | 5.4 | 0.032 | 0.056 | 1.7 | 5.2 | 0.025 | 0.031 | 1.2 |
| 5.1 | 0.032 | 0.080 | 2.5 | 5.1 | 0.029 | 0.052 | 1.8 | 4.3 | 0.018 | 0.023 | 1.3 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5.0 | 0.030 | 0.078 | 2.6 | 4.9 | 0.024 | 0.049 | 2.0 | 5.4 | 0.015 | 0.033 | 2.2 |
| 4.7 | 0.035 | 0.073 | 2.1 | 4.9 | 0.031 | 0.049 | 1.6 | 4.8 | 0.024 | 0.027 | 1.1 |
| 4.8 | 0.033 | 0.074 | 2.3 | 5.1 | 0.028 | 0.052 | 1.8 | 4.4 | 0.020 | 0.024 | 1.2 |
| 4.8 | 0.032 | 0.074 | 2.3 | 4.9 | 0.030 | 0.049 | 1.6 | 4.8 | 0.021 | 0.027 | 1.3 |

Bristol

| T80M | P80M | Gest | Ratio | T90M | P90M | Gest | Ratio |
|---|---|---|---|---|---|---|---|
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| 7.3 | 0.034 | 0.044 | 1.3 | * | * | * | * |
| 7.3 | 0.035 | 0.044 | 1.3 | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |

Rocket

| T10M | P10M | Gest | Ratio | T20M | P20M | Gest | Ratio | T50M | P50M | Gest | Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.4 | 0.053 | 0.232 | 4.3 | 7.4 | 0.049 | 0.216 | 4.4 | 7.2 | 0.038 | 0.181 | 4.7 |
| 7.2 | 0.051 | 0.224 | 4.4 | 7.2 | 0.048 | 0.209 | 4.4 | 6.9 | 0.038 | 0.171 | 4.5 |
| 7.3 | 0.052 | 0.228 | 4.4 | 7.3 | 0.048 | 0.213 | 4.5 | 7.1 | 0.034 | 0.177 | 5.3 |
| 7.3 | 0.051 | 0.228 | 4.4 | 7.3 | 0.048 | 0.213 | 4.4 | 7.2 | 0.040 | 0.181 | 4.5 |
| 5.1 | 0.034 | 0.146 | 4.3 | 5.1 | 0.029 | 0.136 | 4.7 | 3.9 | 0.014 | 0.087 | 6.0 |
| 5.3 | 0.034 | 0.153 | 4.5 | 5.2 | 0.031 | 0.139 | 4.6 | 4.6 | 0.021 | 0.104 | 4.9 |
| 5.3 | 0.032 | 0.153 | 4.7 | 5.2 | 0.026 | 0.139 | 5.4 | * | * | * | * |
| 5.2 | 0.032 | 0.149 | 4.6 | 5.2 | 0.027 | 0.139 | 5.1 | * | * | * | * |
| 4.9 | 0.031 | 0.139 | 4.5 | 5.1 | 0.026 | 0.136 | 5.2 | * | * | * | * |
| 5.1 | 0.028 | 0.146 | 5.1 | 4.6 | 0.023 | 0.121 | 5.4 | * | * | * | * |
| 4.7 | 0.032 | 0.133 | 4.1 | 5.0 | 0.027 | 0.133 | 4.9 | * | * | * | * |
| 4.8 | 0.031 | 0.136 | 4.3 | 4.8 | 0.025 | 0.127 | 5.1 | * | * | * | * |

Rocket

| T80M | P80M | Gest | Ratio | T90M | P90M | Gest | Ratio |
|---|---|---|---|---|---|---|---|
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |
| * | * | * | * | * | * | * | * |

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**G.R. Squire, B. Marshall, G. Dunlop, G. Wright; Genetic basis of rate-temperature characteristics for germination in oilseed rape, Journal of Experimental Botany, Volume 48, Issue 4, 1 April 1997, Pages 869–875, https://doi.org/10.1093/jxb/48.4.869**

**B. Marshall, G. Dunlop, G. Ramsay, G.R. Squire; Temperature-dependent germination traits in oilseed rape associated with 5'-anchored simple sequence repeat PCR polymorphisms, Journal of Experimental Botany, Volume 51, Issue 353, 1 December 2000, Pages 2075–2084, https://doi.org/10.1093/jexbot/51.353.2075**